U.PORTO

FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO

DISSERTAÇÃO DE CANDIDATURA AO GRAU DE DOUTOR

ROOTS OF EARLY OBESITY

ANXIETY, ATTACHMENT AND NEUROENDOCRINE BIOMARKERS IN OBESE CHILDREN

INÊS CRISTINA DE OLIVEIRA PINTO

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2017

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"Love and compassion are necessities, not luxuries.
Without them humanity cannot survive."

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Abbreviations and Acronyms

ADHD	Attention-Deficit Hiperactivity Disorder
ACTH	Adrenocorticotropic Hormone
AVP	Arginine Vasopressin
BED	Binge Eating Disorder
11β-HSD	11-beta Hydroxysteroid Dehydrogenase
BSC	Biological Sensitivity to Context
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
CBCL	Child Behaviour Checklist
CRH	Corticotrophic Releasing Hormone
DMM	Dynamic Maturational Model
DSM-5/IV	Diagnostic and Statistical Manual of Mental Disorders 5th edition/ 4th edition
EADS-C	Escala de Ansiedade, Depressão e Stresse para crianças; (Anxiety, Depression and Stress Scale, child version)
EADS	Escala de Ansiedade, Depressão e Stresse para adultos; (Anxiety, Depression and Stress Scale, adult version)
EEG	Electroencephalogram
Epi	Epinephrine
FACES	Family Adaptation and Cohesion Scales
GC	Glucocorticoid
Glucose	Basal Plasma Glucose
GxE	Gene-Environment
GR	Glucocorticoid Receptor
HDL	High Density Lipoprotein Cholesterol
HPA-axis	Hypothalamic-Pituitary-Adrenal Axis
HPT-axis	Hypothalamic-Pituitary-Thyroid Axis
ICD	International Classification of Diseases
IVIA	Inventário sobre a Vinculação na Infância e Adolescência, hetero-avaliação; (IACA (parent-report) – Inventory of Attachment in Childhood and Adolescence)
LDL	Low Density Lipoprotein Cholesterol
Max	Maximum
Min	Minimum

MR	Mineralocorticoid Receptor
MS	Metabolic Syndrome
n	Number of assessed children; number of children per correlation
NE	Norepinephrine
p	Significance Level
P	Percentile
PVN	Paraventricular Nucleus of the Hypothalamus
PTSD	Post Traumatic Stress Disorder
R^2	Discriminant Coefficients
r_s	Spearman Correlation Coefficient
SAM	Sympathetic Adrenomedullary
SD	Standard Deviation
SNS	Sympathetic Nervous System
TSH	Thyroid-Stimulating Hormone
Type A	Insecure-Avoidant Attachment
Type B	Secure Attachment
Type C	Insecure-Resistant Attachment
Type D	Disorganized Attachment
VMH	Ventro-Medial Hypothalamus

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Summary

Introduction: The quality of the relationship between child and his primary caregiver affects the child's neurodevelopment, emotion regulation, and stress response. Extreme or sustained stress responses are associated with dysregulation of physiologic systems involved in emotion and energy balance, which could lead to emotional and/or behavioural disorders, and obesity. These stress responses can be both physiologic (eg, altered cortisol levels) and behavioural (eg, increased food consumption, internalizing/externalizing symptoms). In our papers we have proposed that insecure attachment and internalizing symptoms may be associated with neuroendocrine biomarkers in a sample of obese children. Attachment security, reflecting the quality of the relationship between mother and child, has the potential to affect the neurodevelopment of physiologic systems regulating emotions and weight. However, few studies have examined the associations between attachment security and internalizing disorders with neuroendocrine biomarkers in obese children. If research confirms the associations between quality of mothering and neuroendocrine biomarkers, mental health and obesity prevention interventions could incorporate more emphasis on the quality of maternal–child relationships. This could be acceptable to parents and would offer additional benefits to children's health and well-being aside from maintaining a healthy weight.

The overall aim of the present thesis is to investigate the nature of the relationship between neuroendocrine biomarkers and mental health using a preexisting sample of obese children and their parents. It aims to illuminate the associations between neuroendocrine biomarkers, and anxiety, and depression, through including assessment of attachment and family functioning as potential intermediate variables in these obese children.

Material and Methods: A non-random sample of 104 obese children (55 boys), mean age 10.9 years (standard deviation 1.76), was recruited from a childhood obesity clinic as part of the "Roots or Early Obesity" study. Obesity was defined as BMI[1] above the 95th age- and gender-specific percentiles. Neuroendocrine biomarkers were measured. Symptoms of anxiety and depression, and attachment strategies were assessed with self and parent-reported questionnaires (EADS; CBCL; IVIA)[1]. Family functioning was assessed

[1] The shorthand acronyms are spelt out in the List of Abbreviations and Acronyms

with parent-reported questionnaires (FACES-III)[2]. Multivariate linear regression analyses were performed.

Results: A significant, negative correlation (r_s=-0.779; p=0.003) between girls' cortisol and their mothers' anxiety symptoms was found, limited to high functioning families. Boys scored significantly higher than girls on mother-reported internalizing symptoms but not on self-report. No association was found between cortisol in children and maternal depressive symptoms.

Type A, avoidant attachment strategies, had significant positive association with TSH[1] levels and negative association with cortisol levels (R^2 = 0.352). Type B, secure attachment strategies, had significant positive associations with both hypothyroidism and BMI percentile (R^2 = 0.541). "Insecure attachment" (Types A, C and D in the Berkeley system) strategies showed some evidence of positive association with TSH[2] (R^2 = 0.250).

Discussion: Whether the association between cortisol levels in obese children and maternal mental health is actually restricted to girls from high functioning families or is due to study limitations, requires further research. The lack of associations between cortisol in children and maternal depressive symptoms, suggests a specific association between cortisol and maternal anxiety symptoms.

These findings suggest that there may be commonalities in the regulation of HPA[2] and HPT[2] axes. Processes involved in development of the Type A attachment strategy appear to be associated with effects on the regulatory mechanisms of the HPA[2] axis.

Conclusions: These results highlight the importance of considering family functioning, parental mental state and gender, when investigating neuroendocrine biomarkers in obese children associated with symptoms of anxiety and depression.

In obese children, different attachment strategies are associated with diverse metabolic profiles. How this may contribute to developing differentiated treatment approaches remains to be explored.

Keywords: Anxiety; Child; Hypothalamo-Hypophyseal System; Object Attachment; Pediatric Obesity; Pituitary-Adrenal System; Stress, Psychological; Thyrotropin.

[2] The shorthand acronyms are spelt out in the List of Abbreviations and Acronyms

Resumo

Introdução: A qualidade da relação entre a criança e o seu cuidador principal afecta o neurodesenvolvimento da criança, a sua regulação emocional e resposta ao stress. O stress agudo extremo ou crónico associa-se à desregulação dos sistemas fisiológicos envolvidos no equilíbrio emocional e energético, o que pode levar a perturbações emocionais e/ou comportamentais, e à obesidade. Estas respostas ao stress podem ser fisiológicas (por exemplo, alteração dos níveis de cortisol) e comportamentais (por exemplo, aumento da ingestão calórica, sintomas internalizantes/externalizantes).

Propôs-se que a vinculação insegura e os sintomas internalizantes podem estar associados a biomarcadores neuroendócrinos numa amostra de crianças obesas. A vinculação segura, relacionada com a qualidade da relação entre mãe-filho, tem o potencial de afectar o neurodesenvolvimento de sistemas fisiológicos que regulam as emoções e o peso. No entanto, poucos estudos examinaram as associações entre vinculação e perturbações internalizantes com biomarcadores neuroendócrinos em crianças obesas. Se a investigação confirmar as associações entre vinculação e sintomas internalizantes com biomarcadores neuroendócrinos, as intervenções na saúde mental e na prevenção da obesidade poderiam dar maior ênfase à qualidade das relações materno-infantis. Isto poderia facilitar a adesão dos pais e oferecer benefícios adicionais à saúde e bem-estar das crianças, para além de manterem um peso saudável.

O objectivo geral da presente tese é investigar a natureza da relação entre biomarcadores neuroendócrinos e saúde mental usando uma amostra preexistente de crianças obesas e os seus pais. Pretende-se esclarecer as associações entre biomarcadores neuroendócrinos, ansiedade e depressão, utilizando a avaliação da vinculação e do funcionamento familiar como possíveis variáveis intermédias nestas crianças obesas.

Material e Métodos: Uma amostra de conveniência de 104 crianças obesas (55 meninos), com idade média de 10,9 anos (desvio padrão 1,76), foi recrutada numa consulta de obesidade infantil, fazendo parte do estudo "Roots of Early Obesity". A obesidade foi definida pelo índice de massa corporal acima do percentil 95 ajustado para idade e sexo. Foram medidos biomarcadores neuroendócrinos. Os sintomas de ansiedade e depressão e as estratégias de vinculação foram avaliados com questionários para pais e crianças (EADS,

CBCL e IVIA)[3]. O funcionamento familiar foi classificado através do preenchimento pelos pais do FACES-III[3]. Foram analizados modelos multivariáveis de regressão linear.

Resultados: Observou-se uma correlação negativa significativa (r_s = -0,779; p = 0,003) entre o cortisol das raparigas de famílias funcionais e os sintomas maternos de ansiedade. Os sintomas internalizantes relatados pelos mães (mas não os auto-relatados) são mais intensos nos rapazes. Não foi encontrada associação entre o cortisol das crianças e os sintomas depressivos das mães.

As estratégias de vinculação insegura do tipo evitante (tipo A) apresentaram uma associação significativa positiva com os níveis de TSH[3] e negativa com os níveis de cortisol (R^2 = 0,352). As estratégias de vinculação segura (tipo B) associaram-se positivamente ao hipotiroidismo e ao percentil de índice de massa corporal, ambas com significado estatístico (R^2 = 0,541). As estratégias de vinculação insegura apresentaram alguma evidência de associação positiva com a TSH[3] (R^2 = 0,250).

Discussão: Não é possível assegurar se a associação entre cortisol sérico nas crianças obesas e sintomas de ansiedade materna se restringe a raparigas de famílias funcionais ou se deve a limitações do estudo. Os achados sugerem uma associação específica entre o cortisol sérico nas crianças obesas e a ansiedade materna. Estes resultados sugerem a existência de factores comuns na regulação dos eixos HPA[3] e HPT[3]. Os processos envolvidos no desenvolvimento das estratégias de vinculação do tipo A parecem associar-se aos mecanismos regulatórios do eixo HPA[3].

Conclusões: É importante considerar o género, o funcionamento familiar e o estado mental dos pais ao investigar as associações entre biomarcadores neuroendócrinos em crianças obesas e sintomas de ansiedade e depressão. Diferentes estratégias de vinculação estão associadas a diferentes padrões metabólicos em crianças obesas. Desconhece-se qual a sua contribuição para o desenvolvimento e diferenciação da abordagem terapêutica.

Palavras-chave: Ansiedade; Criança; Sistema Hipotálamo-Hipofise-Adrenérgico; Vinculação; Obesidade Pediátrica; Stress, Psicológico; Tirotropina.

[3] Os acrónimos são discriminados na Lista de Abreviaturas e Acrónimos

Introduction

Introduction

The quality of the relationship between child and his primary caregiver affects the child's neurodevelopment, emotion regulation, and stress response (Kerns, et al., 2014). Extreme or sustained stress responses are associated with the dysregulation of those physiologic systems that are involved in emotion and energy balance, which could lead to emotional and/or behavioural disorders and to obesity. These stress responses can be both physiologic (eg, altered cortisol levels) and behavioural (eg, increased food consumption, internalizing/externalizing symptoms) (Del Giudice, et al., 2011).

Attachment security, as it relates to the quality of the relationship between mother and child, has the potential to affect the neurodevelopment of physiologic systems regulating emotions and weight. However, few studies have examined the associations between attachment security and internalizing disorders with neuroendocrine biomarkers in obese children.

In our papers (see Chapter IX) we have proposed that insecure attachment and internalizing symptoms may be associated with neuroendocrine biomarkers in a preexisting sample of obese children. If further, independent research confirms associations between quality of mothering and neuroendocrine biomarkers, then, mental health and obesity prevention interventions should come to incorporate more emphasis on the quality of maternal–child relationships. This should be acceptable to parents, as they want the best for their child; moreover, it would offer additional benefits to children's health and well-being aside from their maintenance of a healthy weight.

In the meanwhile, we are learning that depression is expected to become the third major cause of morbidity in the world around 2030 (Mathers & Loncar, 2006). Moreover, we are also on the path of an increasing awareness that mental illness can enhance the impact of physical illness, as happens with the metabolic syndrome – highly relevant given the focus of this thesis. It is associated with reduced life-expectancy, comparable to the reduction associated with smoking and, to a lesser extent, to obesity. It may be that the mechanisms for the effect concerns the changes found in inflammatory processes and changes in hormones, like cortisol levels, associated with accompanying stress. Additionally, a negative impact on the immune system function is also observed. A more precise measure of the effect is achieved, when we consider patients with cardiovascular disease, in that the rates of hospitalization and death for those with mental health problems

are up to three times higher. Similar results are found for asthma and diabetes (Mathers & Loncar, 2006).

Worldwide, at least one third of all families can be expected to include someone who is currently mentally ill (NIMH.NIH.gov; 2015). If we focus on individual adults, the figure is 18%, and for children it is 20%. This compares with over a third of adults suffering from long-term physical conditions such as cardiovascular disease (including blood pressure), respiratory disease, musculoskeletal problems or diabetes (NIMH.NIH.gov; 2015). To be sure, there is of course a substantial overlap between physical and mental illness, so that about a third of people with physical illness would also be diagnosed with mental illness. The degree of disability imposed by depression is 50% higher than that for angina, asthma, arthritis or diabetes (NIMH.NIH.gov; 2015).

Obesity is fast becoming the scourge of our time. It is one of the biggest contributors to death and disease in the industrialized world. Meanwhile, as this situation unfolds, it is becoming clear that the pathophysiology of obesity is vastly more complicated than that of a simple balance equation of the "energy in minus energy out" type. There is actually a complex combination of genetics, parenting, psychopathology and life-style factors, influencing diet and energy metabolism that must be taken into account.

In mental health, as well as in obesity, there is a massive unmet need and there are new treatments/approaches which are beginning to be rolled out. Put together, both these circumstances point to the growing perception of an urgent need to rethink the ways in which mental illness and physical illness interact.

Along with this same line of considerations, one may observe that exposure to stress hormones during the perinatal period has an impact on the developing brain structures subsequently involved in cognition and mental health. Concurrently, one may also observe that perinatal attachment programming of Hypothalamic Pituitary Adrenal (HPA) axis function is hypothesised to influence the risk of developing mental illness, cardiovascular and metabolic diseases (Gander, et al., 2015).

Currently there is mounting evidence for the deleterious consequences of severe childhood obesity on multiple domains of functioning and across many developmental periods. These consequences are seen in physical and motor development, including somatic complaints and delayed motor development; they are also seen in social and emotional development, especially in internalizing and externalizing disorders, and in

cognitive and academic development, including developmental delays and lower academic performance (Bass, et al., 2015). Finally, it should also be taken into consideration that other, independent studies of child and adolescent internalizing and externalizing disorders have shown that factors such as attachment, parental psychopathology, family functioning and the activity of the physiological stress-axis have an aetiological importance of their own (Ein-Dor, et al., 2016; Warren, et al., 1997).

Aims and Outline of the Thesis

Aims

The overall aim of the present thesis is to investigate the nature of the relationship between neuroendocrine biomarkers and mental health using a sample of obese children and their parents. As such, it aims to illuminate the associations between neuroendocrine biomarkers, and anxiety and depression, through the inclusion of both assessment of attachment and family functioning, as potential intermediate variables in these obese children.

To the best of our knowledge, the relation between HPA-axis, anxiety and depression (which often co-occur), attachment strategies and family functioning in obese pre-pubertal children has not yet been systematically investigated in such a way.

Outline of the Thesis

In Part I, the first three chapters, the theoretical-empirical component of this research is elaborated. Chapter I concerns childhood obesity, its clinical characterization and aetiology. The results of the empirical studies concerning the relationship between internalizing and externalizing disorders in childhood obesity are analyzed. This chapter concludes with an analysis of the associations between metabolic syndrome and HPA axis functioning.

In Chapter II, the literature review addresses internalizing and externalizing disorders and their relationships with neuroendocrine biomarkers in childhood. Internalizing and externalizing disorders in childhood are described in terms of their clinical manifestations, epidemiology and comorbidity. Then I present the variables influencing internalizing disorders - their development, maintenance and possibilities for change. In the final part of this chapter, an analysis is offered, on the relationship between HPA axis function and internalizing disorders in childhood.

Chapter III addresses attachment and its relation with neuroendocrine biomarkers and psychopathology. In this chapter, theoretical models concerning the development of attachment are described. The main measures for attachment at different ages are presented. Empirical studies about the relationships between the intergenerational transmission of attachment and psychosocial functioning in childhood are mentioned. Finally this chapter presents information on the relationship between attachment strategies and neuroendocrine biomarkers.

Part II includes the empirical studies of this thesis. Chapter IV describes general methodology concerning the data collection.

Chapter V and VI present the results of the studies about the associations between anxiety, depression, attachment strategies, family functioning and neuroendocrine biomarkers in obese children. In these chapters, specific objectives and methodology are described according to the characteristics of the sample. The evaluation measures and the procedures used to collect the data are presented.

Discussion of results is the subject matter of Chapter VII. They are interpreted according to the theoretical models that underlie the present investigation and the empirical studies previously described. Then, the strengths and limitations of the work are presented and subjected to their due critique. Finally, in Chapter VIII, the results of this critique evaluation are used to formulate their potential application, in order to strengthen the core of some future program for obesity reduction in populations of youngsters.

PART I

THEORETICAL-EMPIRICAL FRAMEWORK

CHAPTER I

CHILDHOOD OBESITY – PSYCHIATRIC PERSPECTIVE

Introduction

Overeating, the chronic reduced control and moderation of how much one eats, can lead to obesity, causing suffering, stigmatization and socio-economic costs. However, obesity is not classified as a psychiatric or behavioural disorder. The International Classification of Diseases (ICD) categorizes obesity as a general medical condition. The Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) does not characterize it as a psychiatric disorder "because it has never been established that obesity is aetiologically associated with a psychological or behavioural syndrome" (APA, 2013). However, Binge Eating Disorder (BED) observed in a large number of obese individuals is classified by the DSM-5 as a psychiatric disorder (Haedt-Matt, et al., 2011). For obese children and adolescents, often victims of bullying, obesity may be associated with psychological and behavioural symptoms.

The Importance of Focusing on Childhood Obesity

Childhood obesity has become a major international public health problem. Some studies, especially those conducted in developed countries but also in developing countries, have consistently shown rising rates of obesity among children, adolescents, and adults. Public funds have been devoted to health education and promotion, in an attempt to reverse this trend.

The prevalence of childhood obesity shows:

- 42 million children under five years of age were overweight worldwide in 2013 (WHO, 2015).
- In Europe, currently, prevalence increases by about 400,000 children each year (the increase has been tenfold since 1970) (Abela, et al., 2014; DGS, 2015).
- In Portugal, over 30% of children are overweight (DGS, 2015).
- There is a deceleration of this growth in high-income countries (Abela, et al., 2014).

- In low- and middle-income countries, malnutrition and overweight persist (Abela, et al., 2014).
- Although the prevalence of overweight is practically the same among girls and boys, the prevalence of obesity is significantly higher in boys (WHO, 2015).
- There are prenatal risk factors for childhood obesity: maternal body mass index (BMI) before pregnancy, maternal smoking during pregnancy and intrauterine effects on subsequent appetite, metabolism and activity levels (Abela, et al., 2014).
- Risk factors identified at 3, 5 and 7 years were: high birth weight, child ethnicity and parental overweight (DGS, 2015).
- Risk factors related to maternal characteristics (poor health, smoking and low schooling), children's eating habits and sedentary lifestyle (DGS, 2015).

Neuroscience of Eating Behaviour and Weight Maintenance

Energy balance and dietary intake are regulated by the hypothalamus. Leptin, an adipocyte hormone, and insulin, present in proportion to body fat reserves, have a high density of receptors in the hypothalamus. The presence of leptin and insulin activates the anorexigenic branch of the hypothalamus (which reduces food intake) and inhibits the orexigenic branch (which stimulates the nutritional contribution). The absence of these hormones activates the orexigenic branch and inhibits the anorexigenic branch, increasing the dietary intake. Additionally, peripherally generated signals such as decreased plasma levels of glucose, cortisol and ghrelin may also increase food intake.

There are other brain regions that influence hypothalamic control and food intake through projections to the ventro-medial hypothalamus (VMH). The dopaminergic brain areas affect eating behaviour and motivation. In the 1960s, weight problems were treated with stimulants, such as dextroamphetamine, that alter intrasynaptic levels of dopamine (Towell, et al., 1988). The identification of anomalies in brain receptors, or in their function, in obese children and adolescents may lead to earlier and more accurate identification of susceptible individuals. Other neurotransmitters involved in food and weight control are serotonin, cortisol and norepinephrine. The serotonergic neurons that project to areas of the VMH, originate in the raphe nuclei. Studies indicate that serotonin induces satiety and decreases dietary intake. The role of cortisol and norepinephrine in

regulating appetite is not yet fully understood. The understanding of appetite regulation by neurotransmitters and hypothalamus will improve the treatment of obesity.

Aetiology

More than 90% of obesity in childhood is of idiopathic aetiology (Buttitta, et al., 2014) and it is this large percentage that is responsible for the childhood obesity epidemic (DGS, 2015; Lazzeri, et al., 2008). Although obesity may be a direct consequence of certain childhood syndromes (eg, Prader-Willi syndrome) or diseases (such as hypothyroidism), these can be related to a small fraction of obese children only (less than 10%), and they will not be considered in this thesis.

Importance of Psychiatric Factors in Obesity: Their Relevance for Research Design

Obesity, in general, does not appear to result from a psychiatric disorder, although weight gain may accompany some psychiatric disorders such as depression. Psychiatric research generally does not assess the temporal relationship between the onset of classic psychiatric disorder and weight gain.

Many population studies have found a high prevalence of psychological problems in obese children and adolescents, especially in females (Mustillo, et al., 2003). Sanderson, et al., (2011), indicate that obese children present higher prevalence of behavioural problems, namely, internalizing problems, than normal weight children do. Depressive symptoms in girls have been associated with concerns about overweight and it goes without dispute that such a latent vulnerability may well be reinforced by the social stigma that overweight/obese children suffer from peers, teachers and even parents.

Some studies have found a significant difference in psychiatric comorbidity among obese children who are referred to the clinic and those of the general population (Puder, et al., 2010). Those seeking clinical treatment have higher levels of depression, anxiety, somatoform and eating disorders (bulimia nervosa, binge eating and anorexia nervosa) (Kovacs, 1985; Griffiths, et al., 2010; Puder, et al., 2010).

Neglect, abuse and, generally, a poorly supporting family environment are factors associated with obesity (Jackson, et al., 2009). According to Lissau, neglected children may be 9 times more likely to become obese (Lissau, et al., 1994). Adults seeking treatment for obesity present a 4-fold higher risk of having been sexually abused in childhood, and twice

as likely to have suffered any other type of abuse compared to control populations (Noll, et al., 2007).

Dysfunctional families can lead to neglect of their children, with a sense of abandonment and no limit on the use of food. Food is hypothesized to provide comfort and serve as a compensation mechanism for children who are victims of traumatic experiences or have lived in difficult environments (Lazzeri, et al., 2008). Thus, children may overeat as a result of environmental deprivation or as a result of depression, somatization or family abuse (Noll, et al., 2007). Some of these children develop a pattern of high caloric intake, becoming severely obese between 3 and 5 years of age. They have a worse prognosis because of the constellation of risk factors with early onset (Tsiros, et al., 2008).

Garthus-Niegel, et al., (2010), suggest that protective and risk factors that influence psychopathology in obese children should be investigated. The high prevalence of psychological problems in obese children and adolescents clarifies the need to measure psychiatric factors, in order to find out whether the current assessment routines should be changed. In this thesis I have had the additional aim of investigating the potential roles played by neuroendocrine factors and attachment strategies – and so their place in assessment routines.

Metabolic Syndrome and HPA Axis Functioning

Obesity is associated with altered HPA activity. The details of this subtle relationship are not known with certainty. One currently accepted hypothesis assumes that the relationship starts with repeated/chronic stress, activation of the HPA and the sympathetic nervous system (SNS) axes, and that insulin resistance, lipid abnormalities and central storage of fat emerge as consequences (Björntorp, et al., 2000). Regardless of the direction of the causal vectors, central adiposity is associated with abnormalities of HPA and SNS function. These neurohumoral abnormalities can explain some of the changes in lipid and carbohydrate metabolism, raised blood pressure and insulin resistance seen in the metabolic syndrome (Bjorntorp, et al., 1992).

The mechanisms underlying the elevation of blood pressure are multiple and include HPA axis hyperactivity. Circulating cortisol levels are higher and the circadian rhythm has its peaks flattened, while glucocorticoid receptor (GR) concentrations in vascular tissue are higher (Bertram, et al., 2002). Cortisol acts directly on the vascular

smooth muscle. Through enhancing accumulation of calcium and its availability to the contractile machinery, cortisol increases its sensitivity to vasoconstriction. Several links seem to exist between the regulation of hypothalamic-pituitary-thyroid (HPT) system and the HPA system (Santos, et al., 2012). Cortisol, a product of HPA-axis activation, inhibits its own release through negative feedback actions at the level of the pituitary and other brain areas. Previous data indicate that glucocorticoids (GC) can inhibit the HPT axis, at the level of the hypothalamus and pituitary (Santos, et al., 2012).

Hypertension, diabetes and dyslipidemia are all regarded as polygenic disorders with genetic contributions of up to a third. However, no clear delineation of the putative influential genes is yet available. Obesity itself appears to be a prime driver or co-traveler of the epidemics of the metabolic syndrome in all populations studied.

In parallel, there is a growing literature on a less obviously genetic modulation of cardiovascular risk arising out of early-life environments. Reports describe a relationship between maternal stress before and during pregnancy, fetal growth, postnatal catch-up growth and risk of cardiovascular disease in later life (Osmond, et al., 2000; Barker, 2002). Epigenetic effects have been proposed as underlying these relationships, both from mother to child and across wider familial and intergenerational distances (Drake, et al., 2005). Methylation of DNA and acetylation of nuclear histones are detailed mechanisms proposed to explain the modulation of gene expression by environmental factors. The epigenetic changes affect children's pattern of ontogeny, growth, response characteristics, metabolic and physiological set-points.

Maternal stress is associated with up-regulation of the maternal HPA-axis, leading to increased cortisol production (Benediktsson, et al., 1993; Lindsay, et al., 2011; Seckl, 2001). Access of maternal cortisol to the fetus is simultaneously enhanced by changes in placental cortisol enzimes (11beta-hydroxysteroid dehydrogenase 2 (11β-HSD2)), which are down-regulated, thereby increasing entry of GC into the fetal circulation. In addition, fetal HPA axis sensitivity is centrally enhanced. Increased fetal exposure to cortisol causes accelerated ontogeny through the process of accelerated differentiation (Fowden, et al., 2004). This results, for example, in a reduced number of nephrons at birth, a reduced pancreatic β-cell mass and shifts in the balance of hepatic enzymes that affect carbohydrate metabolism, promoting gluconeogenesis at the expense of glycogenesis (Curhan, et al., 1996; Langley-Evans, et al., 2001; Ozane, et al., 2002; Moritz, et al., 2005).

If stress-induced changes in mitochondria contribute to the constellation of disorders known as metabolic syndrome, it should be expected that abnormal mitochondrial dysfunction be associated with this syndrome, namely, in its hypertension, insulin resistance, dyslipidaemia, obesity and endothelial dysfunction manifestations. Clinical studies show that this is indeed the case (Ballinger, et al., 2002; Lamson, et al., 2002; Wilson, et al., 2004). Additionaly, several learning disorders and behavioural abnormalities are associated with dysfunctional mitochondria (Aliev, et al., 2003; Cardoso, et al., 2004). This may of course be merely a consequence of cellular dysfunction in general, but there are recent clinical and experimental reports showing that mtDNA mutations are linked to, and precede the development of diabetes and hypertension in humans, implying a causal link (Song, et al., 2001).

There is also evidence from models of developmental programming that, in these circumstances, both mitochondrial function and mtDNA are altered (McConnell, et al., 2003; Park, et al., 2003; Taylor, et al., 2005). It is surprising that very different maternal stressors should all produce changes in mtDNA. These unexpectedly similar outcomes from diverse stressors may suggest that mitochondria are a common vector through which different stressors permanently reset the mitochondrial inheritance of cells.

Significant evidence therefore exists to suggest that a deficiency in placental 11β-HSD2, resulting in the increased exposure of the fetus to maternally derived GC, can programme the HPA-axis and influence postnatal endocrine dysfunction.

According to the Barker hypothesis (Barker, 2002), maternal stress before and during pregnancy is directly related to fetal growth and birth weight of the newborn. Smaller size at birth is related to higher blood pressure and risk of hypertension, and this increase in risk is probably mediated through programmed susceptibility to adiposity and its physiological effects. An interlocking cascade of other metabolic and physiologic mechanisms produces insulin resistance and impaired glucose tolerance, hypertriglyceridaemia and low high density lipoprotein cholesterol (HDL). Together with raised blood pressure they constitute the metabolic syndrome.

In patients with type 2 diabetes, glucocorticoid secretion has been suggested to be a possible link between insulin resistance and the features of the metabolic syndrome (hypertension, obesity, coronary heart disease, hyperlipidemia, and type 2 diabetes) (Chiodini, et al., 2007). The presence of chronic complications of type 2 diabetes (i.e., macroangiopathy, retinopathy, and neuropathy) has been associated with HPA axis activity,

and an association between the degree of severity of several clinical measures of diabetes and cortisol secretion in type 2 diabetic subjects with abnormal HPA activity has been recently reported (Chiodini, et al., 2007).

It seems that the pathogenesis of cardiovascular disease and type 2 diabetes cannot be fully understood within a model in which risks are associated with adverse influences at different stages of life adding to each other. Rather, disease is the product of branching paths of development in complex systems. The branchings are triggered by the environment (Osmond, et al., 2000; Barker, 2002). The specific pathways actually followed determine the vulnerability of each individual to what lies ahead.

This chapter shows that the list of adult diseases whose origins lie in early development has now extended beyond cardiovascular disease and type 2 diabetes. Underlying the association between early growth and later disease, there are also processes which are likely to affect the wellbeing of people who are, from a medical viewpoint, healthy. For example, people who were small at birth have heightened stress responses, one manifestation of lifelong settings of hormones and metabolism that are established before birth.

There is one impressive inequality in the way mental illness is treated, as compared with physical illness. Mental illness is nearly as prevalent as all physical illnesses put together. Mental illness is generally more debilitating than most chronic physical conditions. On average, a person with depression is at least 50% more disabled than someone with angina, arthritis, asthma or diabetes (NIMH.NIH.gov; 2015). Mental pain is as real as physical pain, and it is often more severe. Yet only a quarter of all those with mental illness are in treatment, compared with the vast majority of those with physical conditions (NIMH.NIH.gov; 2015).

Despite converging evidence on the negative consequences of childhood obesity, many questions remain to be addressed. Childhood obesity occurs more often in environments characterized by low socioeconomic status and inadequate family functioning (Dockray, et al., 2009). Across the world, many children continue to live in these risk-increasing environments, enhancers of their eventual vulnerability to psychiatric disorders, to obesity and to an altered HPA-axis activity. Consequently, laboring within such a context, this research has included measures of internalizing and externalizing

symptoms, attachment, family functioning and neuroendocrine biomarkers in order to better address its problematic and complex manifestations. In the next chapters a path will be followed, through these different aspects connected to obesity, emphasizing their relevance for research design.

CHAPTER II

INTERNALIZING AND EXTERNALIZING DISORDERS IN CHILDHOOD

The Relevance of Physiological and Psychological Processes

Overview

Levels of anxiety and depression are high with childhood obesity (Anderson, et al., 2011; Wehry, et al., 2015; Esposito, et al., 2014). In parallel with this, an increasing body of evidence links physiological stress regulation to the aetiology of anxiety and depression (Kagan, et al., 1987). Meanwhile, the mechanism of chronic psychological stress in the causation and progressive development of metabolic syndrome remains to be clarified. It is a reasonable hypothesis to assume that an hedonic response to high fat food could result from self-containment, social isolation and children's inability to properly modulate emotional distress in present human societies. This leads to the suggestion that difficulties in regulating negative emotions, such as fear, sadness, anxiety, and anger, might trigger some compulsive eating response in the absence of hunger (Anderson, et al., 2011).

Internalizing and Externalizing Disorders in Childhood:

Their Relevance for Research Design

The most common forms of childhood psychopathology have been classified into two broad classes of disorders: internalizing and externalizing.

Externalizing disorders are characterized as challenging for relationships. Motivated by somatic feelings and lacking clarity and confidence in predicting what will happen next, individuals focus on feelings as guides to behaviour.

Internalizing disorders involve a range of changes in emotions and mood, namely shame, fear, inhibition, sadness and social isolation. Individuals organize themselves around expected outcomes; they minimize their awareness of feelings, they tend to do what they think that will be helpfully responded to and they tend to avoid doing what they think will be subject to punishment (Crittenden, 2006).

According to the American (DSM-5) and World Health (ICD-10) classification systems, internalizing disorders concern anxiety and mood disorders and externalizing disorders disruptive or hyperkinetic disorders.

Anxiety disorders are characterized by increased physiological activation, excessive preoccupations and avoidance as a way of dealing with the situation. High arousal is consistent with the preparation of the body for self-protective action even when the source of danger is unknown. Mood disorders are characterized by persistent sadness, anhedonia, annoyance, irritability, disturbances in sleep and in appetite (APA, 2013).

There is substantial comorbidity between internalizing and externalizing disorders. Axelson and Birmaher (2001) showed high comorbidity between anxious and mood disorders: 25 to 50% of children with depression presented anxiety disorders and 10 to 15% of children with anxious disorders reported depression. Other studies showed significant levels of comorbidity between conduct and opposition disorders and attention-deficit hiperactivity disorder (ADHD), mood disorders (Capaldi & Stoolmiller, 1999) and anxious disorders (Maughan, et al., 2004). Thus this research has addressed comorbidity when examining whether associations were specific for anxiety and depression, or applied to the broader dimension of internalizing and externalizing disorders.

Current diagnostic procedures tend to focus the clinician's attention on the formation and analysis of symptom clusters and, thereby, to emphasize to a lesser extent the role of matters as important as the aetiology of the disorder and the context of its display. In the dynamic maturational model (DMM) of attachment and adaptation (Crittenden, 2006), the strategic role played by symptoms in a caring relationship highlights their functionality (Wilkinson, 2003), albeit chance connections between symptoms and events can lead to superstitious behavior as the information about a connection between symptom and events (including changes in body states) is. This thesis aims to address symptoms following the DMM of attachment and adaptation (Crittenden, 2006).

Recognizing that behaviour results from the process of mental representation helps to understand why individuals exposed to similar dangers can have different outcomes. The same can be said about genetically identical individuals when exposed to different threats. The representational process is what organizes the individual's behaviour, rather than genes or experience directly. In fact, there is little evidence that genes alone are sufficient to cause mental illness, nor is it evident that they are an essential condition. To the contrary, more often, genetic influence functions solely as a contributing factor. The same can be said

about experience, in that it does not determine outcomes in an inexorable way. Attachment theory, which will be addressed in the next chapter, through its emphasis on individual representation of events, suggests a process by which similar circumstances could yield different outcomes. Both representation and strategic action are treated as being the interactive outcome of universal maturational processes, individual genetic differences, and unique environmental contexts.

Children are at particular risk for over-attributions of danger and maladaptive responses because of their cortical immaturity and their greater vulnerability to danger. Given the chance to reflect over life, maturation and development facilitate the correction of these errors, except in cases where severe, on-going, and deceptive danger may hinder reflective processes. The pervasive and ambiguous nature of threat increases the probability of incompletely processed information regulating behaviour. Together with increasingly precortical distortions of representations, the outcome in adolescence or early adulthood can manifest itself in a variety of complexly distorted patterns of behaviour.

The Importance of Focusing on Childhood Internalizing Disorders

Anxiety and depression disorders are referred to as the most prevalent among internalizing disorders, both in childhood and in adolescence. The prevalence of anxious disorders ranges from 2.8% to 17.7%, with higher rates in clinical samples ranging from 27% to 45% (Martin, et al., 2007).

Evidence is accumulating that disruptions to key neurobiological systems characterize at least some children and youth with internalizing disorders. One of the primary reasons for internalizing disorders being a major public health problem, with significant costs to individuals and to society, is that they are often chronic, recurrent, and increasingly harmful over time (Judd, 1997). In particular, when depression arises during adolescence the condition is far more likely to be associated with a persistent, pernicious course of an illness condition over the lifespan (e.g., Lewinsohn, et al., 1999). For example, as many as 84% of depressed youths will relapse (Harrington, et al., 1996) and these individuals are at particularly high risk for developing a wide range of psychiatric and physical health problems in later adulthood (e.g., Achenbach, et al., 1995).

There is a research imperative to begin mapping possible changes in HPA axis functioning among anxious and depressed individuals at the transition to adolescence. When compared with childhood, it has been well established that adolescence is marked by

significant and relatively abrupt increases in stressful life events (Ge, et al., 1994), particularly within the interpersonal context (Rudolph & Hammen, 1999). Furthermore, it has been found that adolescent girls are exposed to a higher number of interpersonal stressors and that they report greater distress in response to them, when compared with younger children and adolescent boys (Hankin, et al., 2007). Taking all this into consideration, this research concentrates on a clinical sample of pre-pubertal obese children, which is to say, a group at a particularly high risk for developing a wide range of psychiatric and physical health problems.

Internalizing Disorders and HPA-Axis Functioning

Although necessary for survival, the impact of physiological stress reactions on the developing brain may be of particular note, helping to explain how adverse rearing experiences heighten the risk of internalizing disorders in children and adolescents (Sanchez, et al., 2001). In the following sections, we briefly outline the anatomy and physiology of stress, what is currently known about the development of stress reactivity and regulation, the social regulation of stress in human development, the impact of maltreatment on stress neurobiology, and the importance of individual differences as a lens through which to approach the present study's topics. Our increasing understanding of the role of HPA-axis functioning in orchestrating responses to psychosocial threats is important when studying links between emotional behaviour and physiological responses to stressors.

Neuroanatomy and Physiology

The HPA system produces GCs which are steroid hormones. Unlike epinephrine (Epi), which does not cross the blood-brain barrier to a significant degree, the brain is a major target of GCs (Bohus, et al., 1982). Also unlike Epi, GCs production takes some time (approximately 25 minutes to peak levels), and many of its impacts on the body and brain occur through the changes in gene expression (de Kloet, 1991). Consequently, the impacts of GCs are slower to develop and continue for longer periods of time (de Kloet, et al., 1996). Regulation of both the sympathetic adrenomedullary (SAM) and HPA systems converges at the level of the hypothalamus, where autonomic and endocrine functions are integrated with behaviour (Palkovits, 1987). Furthermore, inputs to the hypothalamic nuclei that orchestrate HPA and SAM responses to psychosocial stressors involve cortico-limbic pathways (Gray & Bingaman, 1996).

The Limbic HPA-Axis

The cascade of events that leads to the production of GCs by the adrenal cortex begins with the release of corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) by cells in the paraventricular nuclei of the hypothalamus (Figure 1). CRH and AVP travel through small blood vesicles to the anterior pituitary, where they stimulate the release of adrenocorticotropic hormone (ACTH) (Stratakis & Chrousos, 1995).

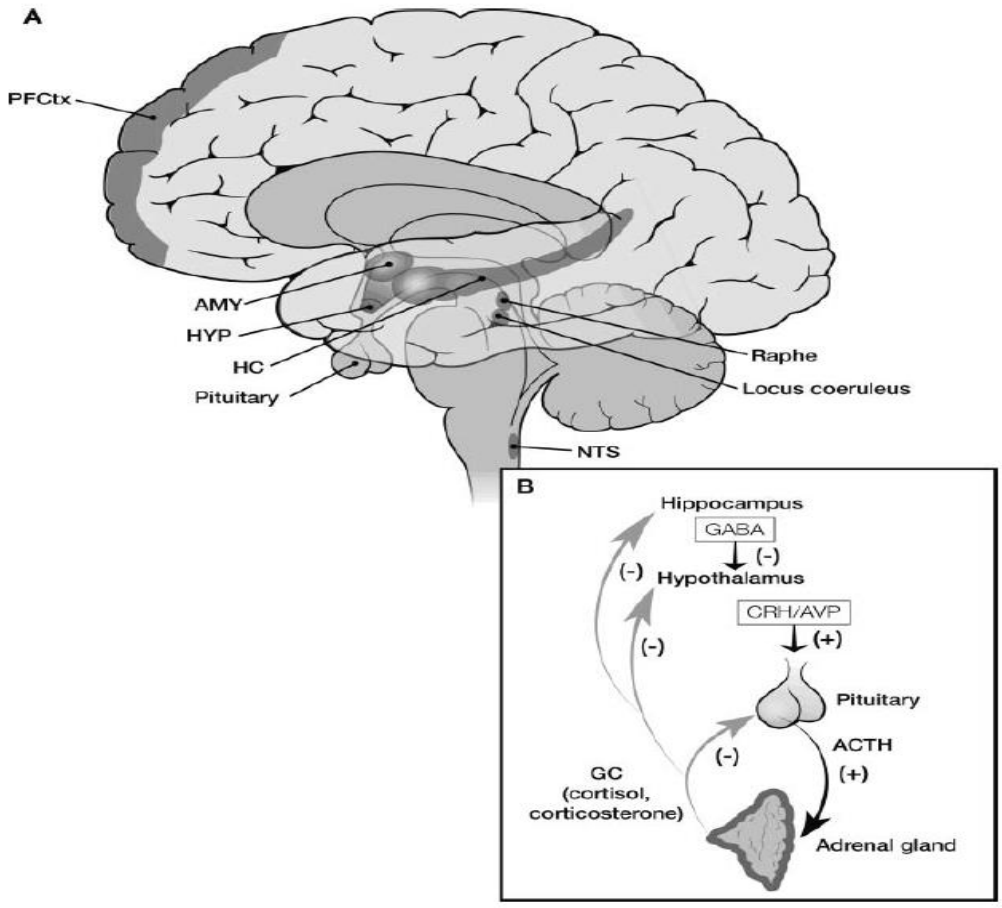

Figure 1: The anatomy of the hypothalamic-pituitary-adrenocortical (HPA) system and the structures that are important in its regulation (Gunnar, 2000).

ACTH interacts with receptors on the cortex of the adrenal gland to stimulate the production and release of GCs into general circulation. GCs enter into the cytoplasm of cells throughout the body and the brain, where they interact with their receptors (de Kloet,

1991). The activated receptors enter the nucleus of the cell, where they regulate the transcription of genes with GC-responsive regions. The action of GCs on target tissues involves changes in gene transcription, which explains why the effects of elevated GCs may take many minutes to hours to be produced and may continue to exert effects on physiology and behaviour for prolonged periods (Sapolsky, et al., 2000).

The effect of GCs depends upon the receptors with which they bind. There are two GC receptors: mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) (de Kloet, 1991). Outside the brain, GCs operate through GRs because of the presence of an enzyme, 11β-HSD that prevents GCs from binding to MRs. In the brain, where 11β-HSD is minimally expressed, GCs bind to both MR and GR. Actually, GCs have higher affinity (i.e., bind more readily) to MRs than to GRs, a fact that is critical in the regulation of both basal and stress responses of the HPA system (reviewed in Gunnar & Vazquez, 2006). Because of their differential affinities for GCs, MRs are 80%–90% occupied when GCs are in basal ranges (de Kloet, 1991). By contrast, GRs are occupied only at the peak of the circadian cycle or when stressors stimulate GC elevations over basal concentrations. GRs mediate most of the stress effects of GCs, whereas MRs tend to mediate most basal effects, which include effects such as maintaining responsiveness of neurons to their neurotransmitters, maintaining the HPA circadian rhythm (highest at wakening and lowest 30 minutes after the onset of the long sleep period each day), and maintaining blood pressure (Sapolsky, et al., 2000). Although these basal effects are often considered distinct from the stress effects of GCs, they play a permissive role in stress. Basal levels allow effective fight/flight responses by allowing norepinephrine (NE) and Epi to have maximal impacts on their target tissues. Presently, it is not known if attachment strategies, i.e., a longer lasting predisposition to react in particular ways, link to basal levels, but it is reasonable to assume that they may prove to be of some relevance in the regulation of both basal and stress responses of the HPA system.

GR-mediated effects often oppose the ones exerted through MR, thereby leading some researchers to argue that stress resilience and vulnerability involve the ratio of MR-to-GR activation (de Kloet, 1991). For example, GRs impair neural plasticity and the processes involved in learning and memory, as evidenced by their impact on hippocampal neurons. By contrast, basal levels of GCs acting via MRs enhance synaptic plasticity, as evidenced by a reduction of the refractory period of hippocampal neurons. MRs facilitate cerebral glucose availability, whereas GRs inhibit glucose utilization throughout the brain, thus endangering cell survival. GRs also activate pathways back to the paraventricular

nucleus of the hypothalamus (PVN), which results in inhibition of CRH production (negative feedback) and thus a termination of the HPA stress response.

Both chronically low and high levels of GCs are associated with non optimal adaptation. In contrast, moderate levels of GCs (or their controlled elevations) are associated with physical and behavioural health.

Psychosocial Stressors: The Role of CRH

The central nucleus of the amygdala and CRH-mediated changes are involved in activating the HPA response to psychosocial stressors (Shekhar, et al., 2005). Pathways to hypothalamic CRH-producing cells that stimulate the HPA cascade are indirect, operating through multisynaptic pathways via the bed nucleus of the stria terminalis that converge on the paraventricular nuclei in the hypothalamus (Herman, et al., 2002). These multiple, converging pathways allow modulation of the strength of the HPA responses in relation to the state of the body, time of day, and current levels of circulating hormones.

Reacting to psychological stressors requires appraisal by higher brain structures such as the cingulate cortex and the orbital/medial prefrontal cortex (Barbas, 1995). Threat appraisal also involves subcortical structures such as the bed nucleus of the stria terminalis and the hippocampus, as well as some further integration by hypothalamic and brain stem structures (Davis, et al., 1997). CRH receptors in all of these regions affect components of stress responding (Bale & Vale, 2004). For example, CRH infused into the locus coeruleus in rodents intensifies anxiety-related behaviours, and neurons in the locus coeruleus are sensitized to CRH after being exposed to psychological stressors (Butler, et al., 1990). Similarly to what is the case with GCs, there are two prominent CRH receptors (CRH-1 and CRH-2), which tend to mediate opposing actions (Bale & Vale, 2004). CRH-1 appears to mediate many of the anxiety-related actions of CRH, while CRH-2 mediates mostly of the stress effects on vegetative functions. Consistent with this distinction, CRH-1 receptors are more abundant in the cortico-limbic pathways that mediate fear and anxiety-related behaviours, whereas CRH-2 receptors are found predominantly in subcortical brain regions (Vythilingam, et al., 2002).

Theories on Internalizing Disorders and HPA-Axis Functioning

Evidence is generally consistent with models that hypothesize that atypical HPA axis functioning precedes the emergence of clinical levels of internalizing disorders and that the HPA axis becomes increasingly dysregulated from child to adult manifestations of

internalizing disorders. Relatively little attention has been paid to the period of late childhood, even though it is a time of developmental alterations in the stress system and heightened risk for mental health problems (Spear, 2000). Furthermore, due to several conceptual and methodological reasons, constraining the state of the art in this field of knowledge, many inconsistencies exist in the relevant findings so far. In order to contribute for a better knowledge of late childhood, this research studied a sample of children within this age period.

A general belief is that anxious individuals are characterized by signs of hyperarousal (Comer, et al., 2004). Hyperarousal is a state of alertness and readiness to respond, involving an activation of the HPA-axis that results in higher cortisol levels. More specifically, Kagan and colleagues (Kagan, et al., 1987) proposed that certain individuals might have an inborn tendency towards hyperarousal of the central nervous system (particularly the hypothalamus and the amygdala) due to a lower threshold for activation. As a consequence, reactivity of the HPA-axis is enhanced, resulting in elevated cortisol levels. These individuals compensate for this state of hyperarousal through withdrawal and avoidance of possible stressful situations, and they may become more fearful to end up in such situations. Withdrawn, avoidant and fearful behaviours are characteristics of an inhibited temperament that has often been associated with anxiety (Dietrich, et al., 2007), and such characteristics may make an individual more susceptible to develop anxiety disorders. Signs of relatively high HPA-axis may therefore be associated with higher anxiety levels.

On the other hand, Feder and colleagues (Feder, et al., 2004) found lower nightime cortisol levels and a slower morning rise in anxious (6- to 12-year-old) children, in comparison with the findings amongst depressed children or controls. Del Giudice and Shirtcliff (Del Giudice, et al., 2011) hypothesized that stressful influences in early life may be associated with higher basal levels of cortisol and that hyper-responsiveness to subsequent minor stressors should provoke frequent elevations in cortisol. The resulting periods of high levels of cortisol are further hypothesized to lead to down-regulation of components of the HPA-system. Another possibility is that under repeated stress, the initial cortisol response becomes modified over the years, so that initial high responses subsequently become lower (Van der Vegt, et al., 2010). Based on both these possibilities, and on the assumption that long periods of stress in early life would also predispose to future vulnerability for anxiety, we infer that anxiety disorders can be expected to appear in association with lower basal cortisol levels.

Stress Neurobiology and Adverse Experience: Parental Neglect and Abuse

Post Traumatic Stress Disorder (PTSD) and depression appear to share hyperactivity of CRH at hypothalamic and extrahypothalamic levels (Heim, et al., 2004). Chronic CRH drive on the pituitary in both disorders appears to result in counter-regulatory down-regulation at the level of the pituitary, leading to blunted ACTH in response to pharmacological CRH challenge tests (Heim, et al., 2004). However, these disorders differ in the sensitivity of feedback regulation of the HPA axis.

Depression among adults is often associated with reduced negative feedback regulation (e.g.,Young, et al., 1991), whereas PTSD appears to be associated with increased negative feedback (e.g.,Yehuda, 2000). As a result, adults with depression often hypersecrete cortisol and exhibit prolonged cortisol elevations, whereas adults with PTSD often hyposecrete cortisol and rapidly return to baseline concentrations following perturbation.

Adult survivors of childhood maltreatment who are free from psychopathology could be seen as resilient (Gunnar & Vasquez, 2006). Given their resilience, perhaps it is not surprising to find that across various studies these adults show evidence of reduced activity of stress neurobiology. For example, the CRH challenge test, which produces blunted ACTH responses in individuals with PTSD and/or depression, produces larger than average responses in resilient adult survivors of childhood maltreatment (Heim, et al., 2001). Because the magnitude of the ACTH response is inversely proportional to the pituitary's chronic or trait-like exposure to CRH (Newport, et al., 2003), these results suggest chronic low CRH production in resilient adult survivors. Similar ACTH results have been obtained in response to psychosocial stressors combined with normal to low cortisol and cardiac responses among resilient adult survivors (Girdler, et al., 2003). Finally, the adrenals of resilient adult survivors also show lower-than-expected production of cortisol to ACTH challenge tests (Heim, et al., 2001).

Child Maltreatment and Stress Neurobiology

It has been hypothesized that traumatized children, initially exhibit complex environmentally induced developmental disorders that later branch toward more specific and adult-like pathologies such as depression and anxiety (Cicchetti, 1996). This complexity is evidenced in the data on the stress physiology of abused children, which are often

difficult and challenging to interpret. For example, sexually abused girls evidence blunted ACTH response in reaction to CRH injections, similarly to adult survivors of childhood abuse with depression or PTSD (De Bellis, et al., 1994). However, enhanced ACTH responses and normal cortisol levels to CRH challenges have also been reported for depressed, abused children, in situations where they are still experiencing adverse home lives (Kaufman, et al., 1997).

Besides physical and sexual maltreatment, other factors are accountable for having an impact on the developing neurobiology of stress. There is increasing evidence that severe neglect also alters the stress neuro-axis (De Bellis, 2005). Children living in orphanages serve as an example. Cortisol levels in orphanage-reared infants and toddlers tend to be low in the early morning and lack the normal diurnal rhythm (Gunnar, 2000). Similar low early-morning levels have also been noted for domestically neglected children soon after placement in foster care (Dozier, et al., 2006, Gunnar & Vasquez, 2006). Moreover, there is also increasing evidence that severe early neglect affects the development of cortico-limbic circuits involved in emotion and stress responding (Glaser, 2000). Presently, it is not clear whether neglect and abuse have different effects on the neurobiological systems that regulate stress and emotional function or whether these effects are comparable.

Indeed, there is some evidence that neglect and various types of abuse, whenever in conjunction with exposure to violence, have cumulative effects; the most profound effects on stress reactivity and regulation, thereafter, are noted for children with the largest cumulative exposures (Cicchetti & Rogosch, 2001).

Risk markers, such as insecure attachment, parental psychopathology, parents indulging behaviour and familial vulnerability also play their role (Pruessner, et al., 1997).

A certain familial vulnerability could underlie an HPA-axis dysfunctioning in children that, in turn, increases their risk for future anxiety disorders. Associations might only appear in individuals with high scores on parental internalizing disorders either due to a threshold effect or due to reduced buffering effects. Therefore, the association of measures of HPA-axis functioning with anxiety disorders might only manifest itself in individuals with a high familial vulnerability for anxiety, e.g. high parental internalizing disorders and isolation. In addition, one can assume that individuals with a high familial vulnerability might not only have an increased risk for future anxiety disorders, but may

also lack resources, like social support, to compensate for possible physiological risk factors. In individuals with a low familial vulnerability, such resources may buffer the effects of physiological factors. Evidence is available indicating that especially morning cortisol levels are at least partially determined by genetic factors (Bartels, et al., 2003). These factors influence CRH secretion patterns, feedback effects of cortisol on central glucocorticoid receptors (Rosmond, et al., 2001), or both. In this way, the sensitivity of an individual to stressful stimuli may be determined, which may increase the risk for anxiety disorders. Other non-genetic familial factors could also be involved. Possibly, parents with high levels of internalizing disorders influence their children's vulnerability to anxiety disorders through controlling and overprotective parenting behaviours (Mcleod, et al., 2007). Such parenting behaviours increase feelings of lack of control and helplessness in the child, much like those which, in their turn, have been associated with abnormal variations of cortisol levels. Together, feelings of lack of control and abnormal variations of cortisol levels might increase the risk for future anxiety disorders.

Possibly, genetic and parenting factors interact. If a child shows more stressed or anxious behaviour due to a genetic vulnerability, the parent might be inclined to be even more controlling and overprotective which in turn again disturbs child cortisol and anxiety levels.

In this thesis, factors such as attachment, parental psychopathology and family functioning were examined when studying associations between internalizing disorders and neuroendocrine biomarkers in a sample of obese children.

Different patterns of attachment have been associated with internalizing and externalizing disorders in childhood and adolescence. However, the different associations between attachment and these disorders require greater empirical foundations. The next chapter will focus childhood attachment and its connection with HPA-axis activity.

CHAPTER III

CHILDHOOD ATTACHMENT

The Relevance of Physiological and Psychological Processes

Overview

The child-parent relationship plays a major role in the child's early life, influencing socio-emotional development, emotion regulation abilities and leading to the development of particular attachment strategies (Bowlby, 1988; Kerns, et al., 2014; Fonagy, et al., 2002; Cassidy, et al., 2013; Crittenden, 2006; Wilkinson, 2003). The quality of the relationship influences the development of both behavioural and metabolic regulation systems as they are activated in stressful contexts (Oskis, et al., 2011).

The present chapter will focus on childhood attachment strategies and their relation with physiological stress regulation. In particular, it will illustrate the ability for the adaptation associated with Type B attachment to enact, and sustain, an healthy emotion regulation behaviour with the potential to protect from overweight and obesity.

Attachment Theory: Its Origins

According to Bowlby (1969, 1973, 1988), early attachment is fundamental for a newborn's survival as it maintains contact and promotes closeness with the primary caregiver. From this perspective, attachment is a motivational system for the search and maintenance of contact with the key caregiver, usually the mother (Ainsworth, 1979; Bolwby, 1969), ensuring survival. This multidimensional system, activated from very early ages/birth, provides newborns and infants with biological foundation and behavioural control processes (Bowlby, 1969, 1973). The development of selective and affective bonds is anchored in neuronal circuits, and endocrine processes (Siegel, 2001) and on primordial mechanisms with deep roots in the evolution of mammals (Hirdy, 2003; Miller & Rogers, 2001). Attachment can thus be defined as a "phylogenetically programmed propensity" to connect with one another that enables the survival of the infant through eliciting the support of an adult to meet the infant's needs (Grossmann & Grossmann, 2003).

Bowlby created his theoretical model with the aim of explaining the individual differences commonly observed in the interactions between babies and caregivers. In this model, children with difficulties in evoking and maintaining contact with their mothers were predicted to be at greater risk of an early death. Children who were able to approach and to follow their mothers, attracting and maintaining their attention, were seen as more likely to survive (Bowlby, 1973). In the meanwhile, Harlow's experiments with rhesus monkeys, in different separation situations, supported the assertion of the relevance and impact, or consequence of early experiences in later social behaviours (Suomi, 1999). These experiments were fundamental for Bowlby's theory, according to which the attachment to a significant person enacts a particularly qualified protection against potential environmental hazards (Bakermans & Kranenburg, et al., 2005).

In the roughly 50 years since its initial formulation by Bowlby, attachment theory attracted a great deal of attention and was the seed for the unfolding of many variants of its academic expression. Currently, there are several versions of attachment theory, the two major ones being, the ABCD model, created by Mary Main, and the Dynamic-Maturational model (DMM), created by Patricia Crittenden (both were PhD students of Mary Ainsworth). The argument of this thesis is to be structured around the premises of the latter one (DMM).

In the DMM, "attachment" stands as a core concept within a theory about survival, as it encapsulates the ideas of survival of an individual, through its ensurance of protection from danger, and of that individual's species, through its finding of a reproductive partner (Crittenden, 2006). As a developmental theory, it is concerned about the interactions between genetic inheritance, maturational processes and person-specific experience that come to produce, or cause the individual differences in strategies that are observed, either when protecting the self and progeny, or when seeking for a reproductive partner. These strategies, i.e., the patterns of attachment, provide both a description of interpersonal behaviour and a functional system for categorizing psychopathology.

Main Concepts

Attachment, Attachment Behaviour and Attachment Behavioural System

Bowlby distinguishes the concepts of attachment and attachment behaviour. Attachment started as referring to a strong, lasting and affective bond, which is supposed to be developed over time, between a child and his primary caregiver. He needs this safe

feeling in order to restore his balance and return to explore the world that surrounds him (Bowlby, 1969). Attachment is also considered an important psychological catalyst for the development of trust, self-understanding and greater social skills (Carvalho, 2007).

The attachment behaviour is any behaviour of the child which leads to and maintains access to the attachment figure, and hopefully elicits a response from that person. This behaviour depends on situational conditions, it is anchored in the child's biology, it has a protection function against possible predators and a predictable goal of seeking proximity in dangerous situations (Bowlby, 1988). Initially, the attachment behaviours are crying, smiling - signaling behaviours and seeking for reference figures proximity - grabbing, and following. They grow in their rich diversity of manifestations and, by the end of the first year of life, they normally achieve a significant, systematic complexity (Bowlby 1969, 1980).

The attachment behavioural system integrates these different types of behaviours into an organized intentional unit that seeks to flexibly respond to environment changes, integrating information and adjusting specific behaviours (Bowlby, 1969). The use of the attachment figure as a "secure base" and a "secure refuge" is reflected in the child's motivation to move away from the attachment figure and explore the environment (Schneider-Rosen, 1990) when all is well. If during exploration the child becomes aware of danger, the exploratory system is "deactivated" and the child initiates attachment behaviours, looking for the attachment figure as a "secure refuge." This allows the child to calm down and once he feels safe, the attachment figure is used as a "secure base" for exploration.

Organized Self-Protective Strategies

In the DMM, patterns of attachment (Ainsworth, Blehar, Waters & Wall, 1978) are conceived of as self-protective strategies. The model proposes that humans have an innate propensity to organize themselves self-protectively and, after puberty, sexually. The strategies are the outcome of the joint processing of two types of information: "cognitive" information about causal relations and "affective" information about the somatic feelings associated with contexts. Cognition is the basis for learning theory whereas affect is tied to arousal, with high arousal often being experienced as anxiety. These two forms of predictive information (temporal and contextual) lead to two basic attachment patterns, Types A and C, respectively. Individuals using a Type A strategy organize around expected outcomes. They minimize the importance of information available in their emotions.

Individuals using a Type C strategy are motivated by somatic feelings. Lacking confidence in what will happen next, they focus on feelings as guides to behaviour. Type B is the integration of the two sorts of information and consists of open, direct, and reciprocal communication of expectations and feelings.

Dispositional Representations

In the DMM, dispositional representations are patterns of neurological activity that dispose individuals to act in some manner (Damásio, 1994). Depending upon whether the representation is based on temporal order or on intensity of stimulation, individuals are disposed to behave based on expected consequences, feelings or integrated access to both. The various representations that are generated may dispose an individual to incompatible responses, leading to the responses described by ethologists as 'displacement activities'. When the estimation of danger is very high, individuals are prompted into action on the basis of the precortical representation that signaled threat and appropriate response most clearly. This representation is likely to be an over-estimation of threat, particularly if life so far has been characterized by much danger. The perception of a threat is processed precortically, and therefore error is not easily discerned or corrected. Thus, such a behaviour risks being maladaptive. When this happens often, it is deemed psychopathological.

As adaptive emotion regulation strategies appear to protect from overweight and obesity (Schlam, et al., 2013), attachment as a source of healthy emotion regulation is increasingly being considered in studies of obesity development. Indeed, some studies showed that children who were exposed to low maternal sensitivity (Rhee, et al., 2006) and showed insecure attachment patterns (Anderson, et al, 2011) were at greater risk of overweight and obesity.

Self-Protective Organizations of Behaviour

Secure attachment (Type B), resistant insecure attachment (Type C) and avoidant insecure attachment (Type A) are three core patterns of attachment (Ainsworth, 1979). The "Strange Situation Process" developed by Ainsworth and her colleagues (1978) analyzes child's behaviours as a response to separation and reunion with the attachment figure. It allowed the identification of different patterns of responses (Table 1).

Table 1 - Types of behaviours observed in 1-year-old children during "Strange Situation".

Classification	Attachment	Sample (%)
Type A	Insecure-avoidant	15
Seeks little mothers' contact, rarely gets angry with her, does not ask for help.		
Type B	Secure	70
Begins to explore, the mother is his secure base. Cries few times. Confident.		
Type C	Insecure- resistant	15
Cries a lot, makes tantrums, doesn't tolerate small separations. Limited exploration, always anxious about access to mother.		

Secure attachment (Type B) is associated with the approach of the child to his mother at times of need, and is interpreted as basic trust attributed to the child based on experience of mother's availability and ability to come with a suitable response. The mother has the child 'in mind' and she is available at times of child stress. Through contingent responsiveness, she enables her child to regulate his state, first, via her direct handling of the child and, subsequently, through enabling her child's self-contained management of his affect – an external process which, thereby, facilitates the internalization of affect regulation.

In contrast, the child of a consistently insensitive and unresponsive mother does not learn to expect his mother to be available in stressful situations and develops an insecure-avoidant attachment (Type A). On the other hand, the inconsistently available mother elicits escalating displays of affect in her child, who forces his mother to respond on the basis of an intermittent reinforcement schedule. However, the child remains ambivalent about close contact, when proffered, as it was only elicited under duress (insecure-resistant attachment, Type C).

The Type A child focuses on the environment at the moment of reunion, ignoring the mother or appearing to approach her but with objects between them, minimizing the distress of potentially not being acknowledged. The reunion behaviour of the Type C child is characterized by anxious contact seeking and clinging, while, at the same time, ambivalently resisting contact with his mother (Ainsworth, et al., 1978).

Type A individuals tend to omit feelings from processing and to act in accordance with expected consequences. Type C individuals do the opposite: they act in accordance with their feelings with little priority to consequences.

These attachment patterns have been confirmed and validated in several studies conducted in different countries, including Portugal. About 65% of children are securely attached according to the ABCD system associated with Main, while about 10% to 15% are resistant and 20% are avoidant (Van Ijzendoorn, et al, 1999). These results were in agreement with those obtained in Portugal by Matos & Costa (2006).

Developmental Pathways

Maturation, combined with experience, enables children to develop new strategies that better represent the relation of the self to context. In infancy, the problems are to find contingencies of the self with others, to share affective states with others and to regulate arousal in order to maintain, for increasingly long periods of time, a state of moderate and attentive arousal. Failure to solve these problems, or to accomplish their inherent search for the desired goals with attachment figures, leads to inhibition or exaggeration of affective displays (i.e., Types A or C strategies). If these don´t succeed in changing the probabilities on parental behaviour, the infant may become non-strategic (i.e., depressed with low arousal, disoriented with high arousal, or disorganized with intrusions of high arousal in an inhibitory strategy). In infancy, these three states manifest themselves in a variey of ways, for instance: either as a sad, withdrawn passivity; either as an aimless agitation without interpersonal focus and, sometimes, with self-stimulation; or, finally, as brief seizure-like losses of control at moments of intense stress from which there is quick recovery.

In the preschool years, the risks involved in these patterns of attachment are that some children will fail to establish relations with non-familial adults and children and that those who choose a strategy of escalation of affect may harm themselves while trying to provoke a response from parents. This is to say that, the extreme Type C patterns carry more immediate and obvious risks than the Type A inhibitory strategies. The risk involved with Type A children is that they will learn that their true negative feelings cannot be communicated in words to parents. Thus the children learn to use language to please the listener rather than to express the self. Type C children are more likely to learn that language can be used to deceive parents, especially about future contingencies. The risk is that they will continue to use action to communicate.

During the school years, the cortex matures in ways that, for the first time, permit children to reflect on their own behaviour. Children who are not helped to do this by their attachment figures will find it increasingly difficult to regulate their own behaviour; this will affect both their inner experience of themselves and their outer experience of relationships with peers. Usually, this failure occurs when the costs of failing to be good are so great that the child relies too heavily on adults' perspectives (Type A); or the consequences are so unpredictable that the child relies too heavily on his or her own perspective (Type C). The former satisfies adults (and goes unnoticed or even praised) whereas the latter upsets adults and results in punishment and referrals to mental health or correction services, where they may well meet doctors and other health-care workers who appear to have an excess probability of having Type A strategies (Wilkinson, 2003).

Attachment Assessment

Attachment researchers have developed a series of age-specific assessments to allow researchers and clinicians to assess individuals' representations and behaviour. All of them differentiate Types A, B, and C (including subclassifications). Moreover, the associated, discourse-based assessments also permit evaluation of integrative capacity or "reflective function".

However, methods for evaluating pre-pubertal children's attachment strategies have not been well developed, with the result that there is little information about any relations between metabolic indicators and attachment strategies in this age group. The hypotheses put forward so far have been developed based on research with younger children (O'Connor, et al., 2007).

Intergenerational Transmission of Attachment Patterns

The DMM addresses change and treats adaptation as a dynamic fit of attachment strategy to context, that is, the relation of the individual's developing strategy to parental contingencies and contextual dangers (Crittenden, 2006). Thus, Crittenden (2000) connects change with the developmental opportunities offered by each maturational-developmental shift or, alternatively, changes in the environment requiring accommodation of the child.

If the parents are not in danger and feel safe, ie their attachment strategies are not being activated, and if they feel and act empowered, ie they exert an influence on their environment, then, they are likely to facilitate the development of a similar attachment strategy in their child. But if there is danger and/or the parents feel threatened, and if they

lack the possibility to change their situation, then, their self-protective strategies may create a threat to their child, forcing him to organize his attachment strategy around this threat. This is particularly problematic when parents have unresolved previous experiences of danger and loss which can trigger unhelpful impulsive responses to their children.

Attachment and Psychopathology

The emphasis on the role of experienced danger permits interpretation of cultural differences in distributions of self-protective (attachment) strategies within a population. The same applies to the prevalence of psychiatric disorders. In fact, given that different cultural groups are the subjects of different histories of past exposure to danger, and that they are also differentiated in their current exposures, one should expect differences in distributions of strategies and disorders tied to strategy among them (Grossmann, et al., 2002).

In most families, mothers are the key caregivers of their children and they shape children's eating habits through feeding and parenting practices. Stress and maladaptive emotion regulation strategies lead to an increased intake of (comfort) food (Birch & Ventura, 2009).

Family Functioning

Baumrind (1993) states that the combination of parental warmth and availability with parental authority is best for the development of the child's affective self-regulation as well as social skills and cognitive abilities. Clearly, such an assertion is coherent with the Type B attachment requirements.

Co-parenting in an adaptive family system entails the parental couple think together about the child. Caregiving is supported by the spousal relationship so that it neither excludes the child, as happens in a disengaged family system, nor it risks so, as it happens in an enmeshed one, that is to say, one with permeable boundaries between spousal and caregiving systems. Adaptive systems are expected to produce securely attached children.

In disengaged family systems, there is no room for the child because parents are too needy of each other or hostile and rejecting towards the child. In this case the child is expected to develop compulsive caregiving, compliance or, when these strategies fail, may withdraw and/or show signs of depression (an awareness that nothing he can do will elicit anything more than rudimentary physical care).

Enmeshed family systems offer a multiplicity of scenarios, all of which involve, ie enmesh, the child in the spousal system while, crucially, concealing the true motives behind adult behaviour from the child, so that he attributes more power and consequence to his own actions than it is actually the case. Silence and deception may be associated with eating disorders (Ringer & Crittenden, 2007).

Characteristic dysfunctional patterns of interaction are found in families with obese children (Cassidy, et al., 2013; Patterson, et al., 2013). For instance, children with difficult temperament and insensitive mothers were found to have significantly higher risk of being overweight or obese during school age (O'Connor, et al., 2007). An analogous situation was reported for children in families with mealtime challenges, maternal distress and family conflicts (Spangler, et al., 1999). Professionals and parents agree that babies seem to have a vocabulary of cries for signaling different situations, such as hunger or pain. Judy Dunn, in her book "Distress and Comfort", reported results of a study showing that a baby left to cry for more than a few minutes settles into a rhythmic crying pattern that can no longer be interpreted by adults (Dunn, 1977). Parents may overfeed difficult children in a misunderstanding of being good parents and in an effort to calm or reduce the intensity of their children emotional manifestations (Tarullo & Gunnar, 2006). In this way, unhealthy regulation with food begins in infancy and could continue throughout the childhood.

Attachment and Neuroendocrine Stress Perspective

Attachment and HPA-Axis Functioning

There is evidence that variance in HPA axis activity varies with weight (Patterson, et al., 2013). Obese children's attachment strategies and associated variation in HPA axis activity have not received much attention.

Cortisol, an hormone of the neuroendocrine system, supports attachment between parents and child. In particular, it is known that cortisol is involved in maternal behaviour and response to the child (Fleming, et al., 1997; Fleming, et al., 1993; Stallings, et al., 2001; Maestripieri, et al., 2001). However, the associations between cortisol and parenting are complex and depend of many factors such as maternal age, previous experience and feeding patterns (Krpan, et al, 2005).

Animal Studies of Early Experience and Stress Neurobiology

Maternal licking and grooming regulate the extent to which GR genes in the hippocampus become methylated (Weaver, et al., 2001). Methylation effectively silences

genes. Licking and grooming reduce methylation of hippocampal GR genes. GR genes determine how many hippocampal GRs an animal will have. Because hippocampal GRs are involved in terminating stress responses of the HPA system, high levels of hippocampal GRs mean efficient control of HPA stress response, whereas low levels mean poor or sluggish regulation, more prolonged stress reactions, and vulnerability to allostatic load over the animal's lifetime (Meaney & Szyf, 2005; Weaver, et al., 2001). This is a powerful example of how stress neurobiology can be programmed by social experiences during sensitive periods of development.

Postnatal Human Development and Stress Biology

It is generally assumed that events, whether they are positive or negative, have less of an effect on structures and circuits that are already well developed than on those that are rapidly developing (Dobbing, 1981). Consistent with the principles of attachment theory (Bowlby, 1969), access to a secure base provided by the attachment figure reduces the probability of HPA/CRH stress reactions that could have long-term consequences on brain development.

Children of mothers who are highly sensitive and responsive exhibit greater left frontal brain electrical activity patterns associated with positive emotionality and approach, whereas those with low-responsive mothers exhibit greater right frontal electroencephalogram (EEG) patterns associated with negative emotionality and fearful, inhibited temperament (Hane & Fox, 2006). In nonhuman primates, greater left frontal EEG asymmetry has been shown to correlate with lower cortisol reactivity to stressors (Kalin, et al., 1998). Higher vagal tone, lower cortisol reactivity to stressors, and greater left frontal EEG patterns suggest that, at least under conditions of supportive care, by the latter part of the first year, the human child enters a period of relative stress hyporesponsivity that may buffer or protect the developing brain and result in a more stress resilient child.

The Role of Caregivers

Mesocorticolimbic and nigrostriatal dopamine pathways contribute to the processing of infant-related sensory cues leading to a behavioural response from caregivers. Studies have shown that mothers exposed to their children's stimuli show an increased fMR scan response in dopaminergic reward brain areas rich in oxytocin receptors (Strathearn, 2011). However, in situations where insecure attachment was clearly the case,

Strathearn saw reduced activation of the mesocorticolimbic dopamine reward system in response to infant face cues, as well as a decreased peripheral oxytocin response to mother-infant contact (Strathearn, 2011).

It appears that only little or no excess adrenocortical activation is observed in a securely attached child when separated from his mother (Oskis, et al., 2011; Spangler, et al., 1999; Tarullo, et al., 2006). The findings in the insecure-avoidant and insecure-resistant attachment groups are inconsistent. In some studies, both insecure groups were found to have elevated cortisol levels (Spangler, et al., 1999), others found increased cortisol levels only for insecure-resistant children (Spangler, et al., 2010). Up to some degree, these inconsistencies may reflect disagreement about how to classify particular behavioural strategies. Nevertheless, it is theoretically coherent to expect elevated cortisol levels in association with Type C attachment, given that those children are exposed to chronic unpredictable stress, due to their parents responding to their distress only intermittently. The above mentioned findings point to cortisol levels being, at least partly, affected by the caregiving environment (Hutt, et al., 2013; Meaney, et al., 2001). Subtyping insecure attachment may help explain these differences in cortisol reactivity, since inter-individual variability in HPA-axis activity appears differentiated, when considering, either Type A, or Type C attachment strategies (Gander, et al., 2015; Kiel, et al., 2013).

There is also evidence that family dynamics, beyond attachment security/insecurity, influence cortisol reactivity in developing children. Naturalistic observations from households of typically developing children (ages 2 months to 17 years) yield evidence that traumatic family events (conflict, punishment, shaming, serious quarrelling, and fighting) are strongly associated with periods of elevated cortisol levels when the child's response to acutely traumatic events is compared with their own levels on less traumatic days in the family (Flinn & England, 1995). There is also evidence that early disruptions in the parent-child relationship may produce increased levels of cortisol, by the preschool years, and that these heightened levels predict increased behavioural and emotional problems in the school-aged child (Essex, et al., 2002). Likewise, social adversity that results in high maternal expression of depressive symptoms, including disrupted patterns of parenting, has been shown to be related to higher and less regulated cortisol activity in school-aged children and adolescents (Halligan, et al., 2004; Lupien, et al., 2000). Additionally, in clinical populations of children with behaviour problems, cortisol increases during a parent-child conflict-discussion task have been found to be associated with dysfunctional parenting attitudes and symptoms of anxiety and depression in the child (Granger, et al., 1996).

Caregivers can prevent, or at least, moderate elevations in cortisol for children even during threatening external events. Responsive caregiving creates conditions for the children to elicit help by expressing negative emotions, without triggering the endocrine component of the stress response. Conversely, when the parenting is inadequate and/or is the source of threat, relationships can be a major source of physiological stress for children (Repetti, et al., 2002).

This chapter aimed to present the main ideas underlying Attachment Theory and its contribution to clinical practice. The potential for the adaptation associated with Type B attachment could be a source of healthy emotion regulation that has the potential to protect from overweight and obesity. Attachment theory focuses on protection and reproduction, as central organizing functions, and on the array of ways that these may be realized as the interactive outcome of universal maturational processes, individual genetic differences, and unique environmental contexts. Its contribution to understanding psychopathology includes a model of functional diagnoses (as opposed to symptom-based diagnoses which have notoriously ignored the phenomena of equifinality and multifinality), development-based hypotheses regarding the relation of childhood experiences to later psychopathology, and an information processing model with implications for treatment.

Overall, this knowledge may have practical implications for the ultimate treatment of childhood obesity, by means of potentially providing a simple, low risk set of intervention strategies to educate and to support mother-infant attachment. As childhood obesity is a major public health problem, with serious long-term consequences for child development and behaviour, these studies may be the first step in developing novel treatments in support of behavioural and psychosocial interventions, with many benefits to harvest in the near future.

PART II

EMPIRICAL STUDY OF ANXIETY, ATTACHMENT AND NEUROENDOCRINE BIOMARKERS IN OBESE CHILDREN

CHAPTER IV

BACKGROUND AND METHODOLOGY OF RESEARCH

Theoretical-Empirical Background

Internalizing and externalizing disorders in childhood obesity are quite frequent and are associated with changes in the children´s metabolic profile and psychosocial functioning. Factors such as attachment, parental psychopathology, family functioning and the activity of the physiological stress-axis have been shown to be differentially related to the development and maintenance of internalizing and externalizing disorders in childhood and adolescence (eg, Warren, et al., 1997). However, the relationship between these factors is addressed only in a few of the studies developed to analyze how the disorders develop and are maintained (eg, Shamir-Essakow, et al., 2005).

The isolated evaluation of individual, cognitive, family or environmental factors, without considering their complex interactions, limits the current research. The collection of information from only one source, usually children and adolescents, can explain some of the difficulties in obtaining consistent findings.

General Methodology

Sample

A convenience sample of children attending the Childhood Obesity Paediatric Unit of a Tertiary Paediatric Hospital in Greater Lisbon, was drawn from pre-pubertal obese children recruited for this project named "Roots of Early Obesity" (Pinto, et al., 2011). The effective sample was based on the children for whom we had detailed records of anthropometric information and behavioural measurements throughout childhood. Exclusion criteria were the use of medication, having already reached puberty during the period of assessment, undergoing mental health intervention or other medical treatment besides those for obesity.

Parental written informed consent was obtained. The project was approved by the Hospital Medical Ethics Committee.

The sample consisted of 104 recruited pre-pubertal children, with mean age of 10.88 years (standard deviation 1.76) and together with their parents provided the data for analysis.

Procedure

The children that agreed to participate in this study were evaluated alone by a technician, according to a protocol. Anxiety, Depression and Stress Scale, child version (Escala de Ansiedade, Depressão e Stresse para crianças, EADS-C) was measured, as well as the metabolic parameters listed in table 2.

A parent of the child, usually the mother, provided socio-demographic data, the child's developmental history and details of the medical history, and parent-report versions of EADS, Inventory of Attachment in Childhood and Adolescence (Inventário sobre a Vinculação na Infância e Adolescência, IVIA), Child Behaviour Checklist, (CBCL) and Family Adaptation and Cohesion Scale (FACES III).

Figure 2 describes the general procedure used for collecting the data from the children and parents.

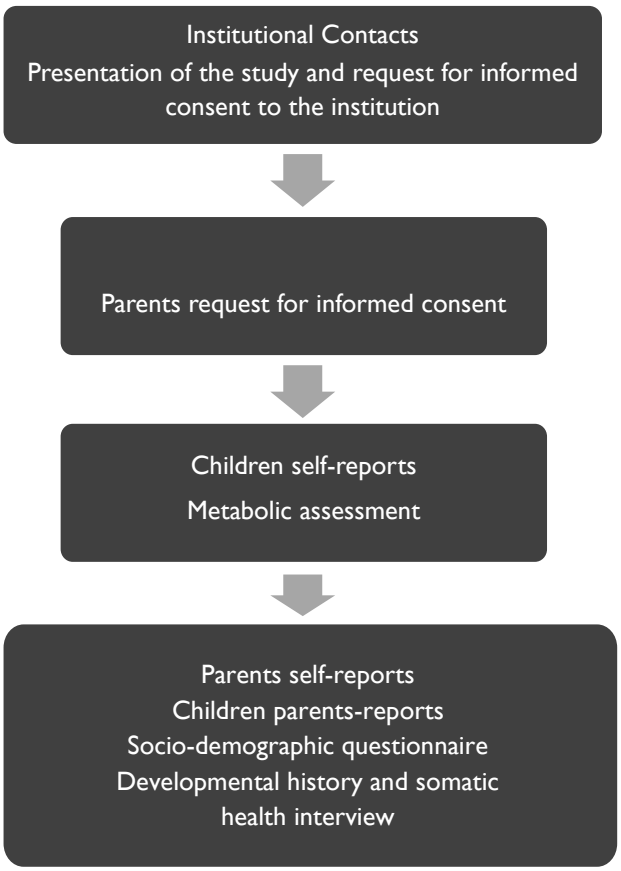

Figure 2 - General procedure for data collection.

Table 2 shows the evaluation matrix used in the present study.

Table 2 – Evaluation matrix of the study.

	Children	**Parents**
Internalizing Problems (anxiety and depression)	EADS-C (self-report) CBCL (parents-report)	EADS (self-report)
Attachment Strategies	IVIA (parents-report)	
Family Functioning		FACES III
Metabolic Assessment	Height, weight, waist circumference, blood pressure and heart rate Plasma cortisol, glucose, insulin, HDL, LDL, triglycerides, catecholamines, ACTH, TSH and T4	
Others	Developmental history and somatic health (parents interview) ex. pubertal stage, medication and perinatal variables, eg pregnancy duration and birth weight	Socio-demographic questionnaire

EADS-C: Escala de Ansiedade, depressão e stresse, child version; EADS: Escala de Ansiedade, depressão e stresse, adult version; CBCL: Child Behaviour Checklist; IVIA – Inventário sobre a Vinculação na Infância e Adolescência; FACES – Family Adaptation and Cohesion Scale; ACTH – adrenocorticotropic hormone; TSH - thyroid-stimulating hormone; HDL - high density lipoprotein cholesterol; LDL - low density lipoprotein cholesterol.

The following two chapters present the specific background, methodology and results.

CHAPTER V

STUDY I: ANXIETY, FAMILY FUNCTIONING AND NEUROENDOCRINE BIOMARKERS IN OBESE CHILDREN

The following publication constitutes part of this chapter:

Pinto I, Wilkinson S, Virella D, Alves M, Calhau C, Coelho R. *Anxiety, family functioning and neuroendocrine biomarkers in obese children.* Acta Med Port. 2017; 30(4): 273-280. [4]

Theoretical Framework

Symptoms of anxiety and depression are frequent in obese children (Anderson, et al., 2011; Wehry, et al., 2015; Esposito, et al., 2014). Anxiety, depression and obesity are recognized human and economic burdens. It is important to investigate the aetiological mechanisms involved (Padula, et al., 2014). Mental disorders, such as anxiety and depression, are associated with decrements in public health that are equivalent to those of other chronic physical diseases (e.g., angina, arthritis, asthma, and diabetes) (Padula, et al., 2014). However, mental disorders are stigmatized and regarded as less important. In fact, it is not generally acknowledged that, when mental disorders coexist with these physical diseases, the decrease in public health scores is substantially greater than when they occur alone. Thus, a crucial implication follows, that primary health care providers should not ignore the presence of depression and/or anxiety disorders when treating patients with a chronic physical disease (Kupfer, et al., 2012).

Metabolic syndrome, of which obesity is a part, shares risk factors with disorders of anxiety and depression. On the other hand, anxiety and depression are also known to increase the risk of obesity. Such a mutually reinforcing relation can contribute to the association between anxiety and depression and coronary artery disease (Kupfer, et al., 2012).

[4] See Chapter IX, Paper I

A possible way to interpret the link between anxiety and depression and obesity is emotional eating. Activation of the stress response can lead to emotional dysregulation that has been associated with increased appetite, a preference for foods high in sugar and fat (Dallman, 2010), fat visceral accumulation and obesity in adults (Torres & Nowson, 2007) and adolescents (Vanaelst, et al., 2012). In this regards some authors revealed that overweight subjects tend to gain weight when stressed (Dallman, 2010) and that obese individuals increase their food intake after having experienced negative emotions and perceived stress (Barrington, Beresford, McGregor, White, 2014). Stress-related adaptation involves the concept of allostasis, which is the ability to achieve the physiological balance through the change of the internal environment (Sinha & Jastreboff, 2013). Conditions of repeated or incontrollable chronic stress are followed by atypical cortisol response and tend to activate a state of allostatic load resulting in neural and emotional dysregulation which contribute to maladaptive behaviours such as repeated consumption of high caloric food (McEwen, 2007), lack of control over eating and binge eating (Groesz, et al., 2012). These results suggest that psychophysiological responses to stress may influence subsequent eating behaviour.

Another possibly potential additional factor promoting the development of obesity in children - maternal mental state - has to be considered. Maternal mental state may directly affect children's risk of becoming obese by fostering stress and emotional arousal in the children themselves. This may lead to an increase in the secretion of stress hormones, which in turn can lead to a greater accumulation of fat in visceral depots (Anagnostis, et al., 2009). Maternal stress may also indirectly affect children's risk of becoming obese due to its adverse influence on health socialization behaviours, e.g., greater maternal reliance on fast food, lower maternal monitoring of children's sedentary behaviour, or an uninvolved maternal feeding style (El-Behadli, et al., 2015). Support for these assumptions is provided by a recent meta- analysis across longitudinal and cross-sectional studies (Tate, et al., 2015) documenting a greater risk of childhood obesity when mothers experienced stress (e.g., financial strain, serious life events, parental worries etc.).

Mothers with symptoms of anxiety and depression and their offspring tend to have similar neuroendocrine biomarkers, such as altered levels of cortisol, peripheral levels of dopamine and serotonin, right frontal electroencephalogram activation and vagal tone (Del Giudice, et al., 2011). If a description of specific subgroups, such as patients in whom certain biomarkers are associated with specific symptoms, was to emerge, then, both short and long term benefits of treatment could potentially be improved. Although new reports

about treatment response in multisite studies have emerged in the past 5 years, treatment advances are lagging due to scarcity of adequate studies with the appropriate indicators of response (Kupfer, et al., 2012).

Since anxiety and depressive problems often co-occur, in an attempt to improve the taxonomy of anxiety and depression, Clark and Watson introduced the tripartite model (Clark & Watson, 1991). According to this model, anxiety and depression share negative affect as a common factor, whereas depression is specifically characterized by low levels of positive affect, and anxiety by physiological arousal. A study by Kovacs and colleagues (Kovacs, et al., 1989) has shown that anxiety precedes the onset of depression in 67% of the comorbid cases. This finding suggests a developmental sequence in the relationship between the two disorders, with subsequent comorbidity emerging first as anxiety. When examining whether associations with neuroendocrine measures are specific for physiological arousal or applied to the broader dimension of internalizing problems, it is important to investigate the role of co-occurring depressive problems.

There is a large amount of research demonstrating that - in addition to normal parental difficulties and life events - specific parental stress is related to children's internalizing behaviours (Rodriguez, 2011) and externalizing behaviours (Mackler, et al., 2015). Again, these links may be established by various mechanisms such as genetic risk factors (Posthuma & Polderman, 2013) and biological pathways, e.g., intrauterinary exposure of the fetus to hormones involved in the stress response (Glover, et al., 2010), but also via parenting behaviour towards the child.

There is consistent agreement that low functioning families (e.g., characterized by harshness, hostility, intrusiveness) contribute to the development and maintenance of child psychopathologies: less warmth, abusive parenting, parental rejection but also over-involvement within the parent–child relationship, all appear to be risk factors for internalizing problems in children (Yap & Jorm, 2015). By contrast, aspects characterizing high functioning families (i.e., greater parental structuring, non-intrusiveness, absence of hostility, and children's involvement of the parents) prior to kindergarten entry predicted lower levels of internalizing or externalizing symptoms in children 1 year later (Biringen, et al., 2005).

Neuroendocrine biomarkers, such as those currently associated with the future development of symptoms of anxiety and depression, seem more frequent in individuals coming from families characterized by anxiety and depression (Wehry, et al., 2015).

Moreover, boys and girls living in such families may show differences in their vulnerability to develop symptoms of anxiety and depression. It is important to take into account the mental state of family members, family functioning and the child's gender, when investigating potential neuroendocrine biomarkers for future anxiety and depression symptoms as found with obesity.

Most strategies for the prevention of childhood obesity are focused on energy balance (Sabin, et al., 2015). They are directed at behaviours that directly affect energy intake or expenditure, such as increasing physical activity, reducing sedentary behaviour, or limiting intake of high energy foods and beverages (Horodyska, et al., 2015). However, the limited success of these strategies (Ciampa, et al., 2010; Hesketh, et al., 2010; Whitlock, et al., 2010) underscores the importance of developing new approaches through identifying other barriers to changing behaviour and hence enabling public health strategies to be more successful.

Given the current state of knowledge, it is reasonable to assume that internalizing symptoms, such as those found with anxiety and depression, may be risk markers for obesity in children (Maniam, et al., 2014; Lee, et al., 2015; He, et al., 2015). Moreover, it also seems sound and acceptable to consider that obesity be mediated through effects on appetite, sleep, and activity (Maniam, et al., 2014; Lee, et al., 2015; He, et al., 2015). The (re)activity of the HPA-system plays a role in the course of disorders of anxiety and depression. In the few studies that have investigated the association between anxiety disorders and cortisol levels in children and adolescents, findings have been as inconclusive as in adults. Del Giudice and Shirtcliff (Del Giudice, et al., 2011) hypothesized that stressful influences in early life may be associated with higher basal levels of cortisol and hyper-responsiveness to subsequent minor stressors provoke frequent elevations in cortisol. The resulting periods of high levels of cortisol are further hypothesized to lead to down-regulation of components of the HPA-system. Another possibility is that under repeated stress, the initial cortisol response becomes modified over the years, so that initial high responses subsequently become lower (Van der Vegt, et al., 2010). Based on both these possibilities, and on the assumption that long periods of stress in early life would also predispose to future vulnerability for anxiety, we infer that anxiety disorders can be expected to appear in association with lower basal cortisol levels.

Given the current lack of information about causal processes leading to the major health risks associated with both anxiety and depression, and obesity, further research is

needed to facilitate the development of both testable hypotheses and the future research agenda.

Objectives and Hypothesis

This study aimed to explore associations between cortisol levels and symptoms of anxiety and depression in obese children. We hypothesized that gender and family functioning, as characterized by different levels of cohesion and adaptability, played a role together with cortisol levels and symptoms of anxiety and depression in our sample. We also considered whether maternal mental state could be associated with family functioning and cortisol levels in obese children.

Methods

Sample and Procedure

A sample of children attending the Childhood Obesity Paediatric Unit of a Tertiary Paediatric Hospital in Greater Lisbon, was drawn from pre-pubertal obese children recruited for the project "Roots of Early Obesity" (Pinto, et al., 2011). The effective sample was based on the children for whom we had detailed records of anthropometric information and behavioural measurements throughout childhood. Exclusion criteria were the use of medication, having already reached puberty during the period of assessment, undergoing mental health intervention or other medical treatment besides those for obesity. Only one parent per child participated, most often the mother.

Parental written, informed consent was obtained. The project was approved by the Hospital Medical Ethics Committee.

Measures

Anxiety and Depressive Symptoms

Anxiety and depressive symptoms were assessed using "EADS-C – Escala de Ansiedade, depressão e stresse para crianças", child-version; and "EADS - Escala de Ansiedade, depressão e stresse", adult-version. EADS-C and EADS are self-report questionnaires validated for Portugal (Leal, et al., 2009; Pais Ribeiro, et al., 2004). The EADS-C and EADS are a set of three self-reported scales (21-items) designed to measure symptoms of depression (dysphoria, hopelessness, devaluation of life, self-depreciation, lack of interest/involvement, anhedonia, and inertia), anxiety (autonomic arousal, skeletal muscle effects, situational anxiety, and feeling anxious) and stress (difficulty relaxing,

hypervigilant, and being easily upset/agitated, irritable/over-reactive and impatient). The responses are classified according to four levels: 0) never happens; 1) happens sometimes; 2) happens very often; and 3) happens almost all the time. EADS-C has a structure similar to the adult version (Pais Ribeiro, et al., 2004) although the load values of the items in each dimension are less discriminative than for adults (Leal, et al., 2009).

The Child Behaviour Checklist (CBCL 6-18) is a parent-reported questionnaire for assessing behavioural or emotional problems in children and adolescents (aged 6- to 18-year-old). It contains 120 items on behavioural or emotional problems experienced in the six months prior to the test. These items are scored on a three point scale: 0 = not true; 1 = somewhat or sometimes true; 2 = very or often true.

The CBCL version used is approved by Achenbach (http://www.aseba.org/ordering/translations.html) (Gonçalves, et al., 2000). This instrument can allocate symptoms to internalizing or externalizing dimensions of function. As conceived by Achenbach (1991), internalizing symptoms refer to three categories: withdrawal, somatic complaints, and anxiety/depression. Externalizing symptoms refer to the categories of delinquent and aggressive behaviour.

Family Functioning

Family functioning was assessed using FACES III (Curral, et al., 1999), a parent-reported questionnaire, validated for Portugal (Curral, et al., 1999). This self-reported 20-item Lykert type questionnaire evaluates two major dimensions (Cohesion and Family Adaptation) on a circumplex model, which is integral to the conceptualisation of functioning employed in FACES. Reliability for Cohesion is 0.78 and for Family Adaptation is 0.70 (Curral, et al., 1999).

Cohesion is conceptualised as the emotional closeness that family members have to one another. Adaptability is conceptualised as the potential for change in family leadership, role relationship, and relationship rules. FACES assesses the degree to which family members are adaptive and connected to each other. The dimension of family adaptability is categorised into four "levels" of functioning: rigid, structured, flexible, and chaotic. The two middle levels (structured and flexible) are considered as balanced family adaptability and the two extreme levels (rigid and chaotic) are considered as unbalanced levels of family adaptability. The dimension of family cohesion is categorised into four "levels" of functioning: disengaged, separated, connected, and enmeshed. Similar to that for family

adaptability, the two middle levels (separated and connected) are considered to be balanced levels of family cohesion and the two extreme levels (disengaged and enmeshed) are considered to be unbalanced levels of family cohesion (Curral, et al., 1999). In other words, balanced levels of cohesion and adaptability are conceptualised as reflecting healthier family functioning, and they are classified as "high functioning families". Unbalanced levels of cohesion and adaptability (very low or very high levels) are seen as reflecting more problematic family functioning, and they are classified as "low functioning families".

Metabolic Assessment

Height, weight, waist circumference, blood pressure and heart rate were measured following standardized protocols. Obesity was defined as BMI ($kg.m^{-2}$) above the 95th age- and gender-specific percentile (WHO Multicentre Growth Reference Study Group, 2006). Overweight was defined as BMI ($kg.m^{-2}$) above the 85th age- and gender-specific percentile (WHO Multicentre Growth Reference Study Group, 2006). Blood pressure was measured three times and the third reading was recorded (WHO Multicentre Growth Reference Study Group, 2006). Age and gender-specific percentiles were used (WHO Multicentre Growth Reference Study Group, 2006).

As part of the "Roots of Early Obesity" project (Pinto, et al., 2011), fasting blood samples were drawn at 8 a.m. through an indwelling catheter, after 45 min of rest. Plasma cortisol, glucose, insulin, HDL and LDL cholesterol, triglycerides and catecholamines were measured using the standard procedures of the Clinical Pathology Laboratory of the institution.

Statistical Analyses

The patient characteristics were described using the mean and standard deviation (SD) or median and interquartile range (IQR: 25th percentile-75th percentile) for continuous variables, as well as frequencies (percentages) for categorical variables.

To investigate the nature of the relation between cortisol levels, and symptoms of anxiety and depression (modelled as ordinal variables), Spearman correlation analyses and significance tests were performed, both with respect to the global sample and to each of its above mentioned subgroups. A level of $\alpha = 0.05$ was considered to be significant. Statistical analysis was carried out using Statistical Package for the Social Science for Windows version 22.0 (SPSS Inc, Chicago IL).

Results

A sample of 104 recruited pre-pubertal children (55 boys), with mean age of 10.88 years (standard deviation 1.76) was included in the analysis. BMI was calculated for 63 children, of whom 59 were obese (35 boys) and 4 were overweight (1 boy).

The values found for the symptom scores (anxiety, depression and internalizing symptoms, which included anxiety and depression in Achenbach's CBCL system) and neuroendocrine and metabolic biomarkers in the complete sample are presented as Tables 3 and 4.

Table 3 - Symptom scores (anxiety, depression and internalizing symptoms) as median (P_{25}-P_{75}) for the whole sample.

Measures	Symptom Scores
EADS Anxiety symptoms n=71	10.0 (2.0-17.0)
EADS Depression symptoms n=71	13.0 (5.0-24.0)
EADS-C Anxiety symptoms n=76	17.0 (2.5-45.5)
EADS-C Depression symptoms n=76	20.0 (6.0-58.0)
CBCL Internalizing symptoms n=65	11.0 (7.0-15.0)
CBCL Externalizing symptoms n=65	9.0 (3.5-16.0)

EADS-C: Escala de Ansiedade, depressão e stresse, child version; EADS: Escala de Ansiedade, depressão e stresse, adult version; CBCL: Child Behaviour Checklist.

Table 4 - The neuroendocrine and metabolic biomarkers.

Parameter (n)	Mean (SD)	Median	Min.- Max.	P_{25} - P_{75}
Glucose (mg/dl) (57)	92.5 (15.4)	93.0	1 – 120	89.0 - 98.0
Insulin (U/ml) (43)	14.55 (9.60)	13.4	0.28 - 52.95	7.99 - 16.92
Triglycerides (mg/dl) (51)	97.21 (39.80)	89.0	33 – 187	68.0 – 129.0
Cholesterol (mg/dl) (53)	161.85 (31.00)	166.0	49 – 227	145.0 - 184.0
HDL (mg/dl) (50)	48.80 (13.87)	47.0	24 – 91	38.0 - 57.3
LDL (mg/dl) (48)	107.93 (23.51)	109.5	59 – 167	95.0 - 118.5
ALT (U/L) (50)	28.20 (23.42)	23.0	11 – 167	17.0 - 30.0
AST (U/L) (48)	24.10 (7.44)	24.5	12 – 56	19.0 - 26.8
TSH (µIU/ml) (56)	2.74 (1.17)	2.6	0.72 - 5.92	1.8 - 3.5
T4 (ng/dl) (53)	1.27 (2.11)	1.0	0.59 - 16.20	0.8 - 1.2
ACTH (pg/ml) (68)	28.89 (20.67)	20.7	5.35 – 118.0	16.3 – 35.2
Cortisol(µg/dl) (53)	10.8 (5.16)	9.95	3.3 - 29.0	7.2 - 13.6

Glucose - Basal Plasma Glucose; Max – maximum; Min.- minimum; n – number of assessed children; P – percentile; SD – standard deviation.

The distribution of symptom scores and serum cortisol levels in the whole sample showed no significant differences between either high and low functioning families, or between boys and girls, but the score (median, P_{25}-P_{75}) for internalizing symptoms (CBCL) showed a tendency to be higher in boys (12.5, 9.0-15.0 vs. 9.0, 6.5-13.5; Mann-Whitney, p=0.066). An interesting observation was that mothers[5] of obese boys scored (median, P_{25}-P_{75}) markedly higher, as close to statistical significance as it is possible to get, for depression symptoms (EADS) compared to mothers of obese girls (28, 10-66 vs. 10, 0-38; Mann-Whitney, p=0.051).

[5] As there were over 90% of participating mothers, the text will reflect the reality of parents being primarily mothers

Table 5A - Correlations of children's symptoms of anxiety and depression.

	EADS-C Depression symptoms		
	Children	**Girls**	**Boys**
EADS-C Anxiety symptoms	r_s =0.644 p ≤0.001 n=76	r_s =0.672 p=0.000 n=43	r_s =0.643 p=0.000 n=33

EADS-C: Escala de Ansiedade, depressão e stresse, child version; r_s: Spearman correlation coefficient; p: significance level, n: number of children per correlation.

Table 5B - Correlations of maternal symptoms of anxiety and depression.

	EADS Depression symptoms		
	Children' mothers	**Girls' mothers**	**Boys' mothers**
EADS Anxiety symptoms	r_s =0.691 p≤0.001 n=71	r_s =0.714 p=0.000 n=37	r_s =0.616 p=0.000 n=34

EADS: Escala de Ansiedade, depressão e stresse, adult version; r_s: Spearman correlation coefficient; p: significance level, n: number of mothers per correlation.

There was a significant, positive correlation between symptoms of anxiety and depression in children (p≤0.001; r_s=0.644), stronger in girls (p=0.000; r_s=0.672) (Table 5A) (EADS-C). Symptoms of anxiety in mothers had a significant, positive correlation with maternal depression, stronger in girls' mothers (p=0.000; r_s=0.714) (Table 5B) (EADS).

Table 6 - Correlations between maternal's symptoms of anxiety and depression and children's symptoms of anxiety and depression.

	EADS-C Anxiety symptoms			EADS-C Depression symptoms		
	Children	**Girls**	**Boys**	**Children**	**Girls**	**Boys**
EADS Depression symptoms	r_s=0.335	r_s =0.332	r_s =0.397	r_s=0.462	r_s =0.706	r_s =0.243
	p=0.017	p=0.097	p=0.055	p=0.001	p=0.000	p=0.253
	n=50	n=26	n=24	n=50	n=26	n=24

EADS-C: Escala de Ansiedade, depressão e stresse, child version; EADS: Escala de Ansiedade, depressão e stresse, adult version; r_s: Spearman correlation coefficient; p: significance level, n: number of children per correlation.

There was a significant, strong positive correlation between mothers' and daughters' symptoms of depression (p=0.000; r_s=0.706). Symptoms of depression in mothers had a significant but low positive correlation with children's symptoms of anxiety (r_s=0.335; p=0.017) and children's symptoms of depression (r_s= 0.462; p=0.001) (Table 6) (EADS, EADS-C). No associations were found between maternal symptoms of anxiety and children symptoms of anxiety or depression.

Serum cortisol levels in girls had a significant (p = 0.026) but low (r_s=-0.464) negative correlation with mother-reported anxiety score (EADS) (Table 7). Nearly significant negative low correlations were found between serum cortisol levels and mother-reported anxiety score (EADS) in children from low functioning families, child-reported anxiety score (EADS-C) in children from high functioning families and the score for externalizing symptoms (CBCL) in girls (Table 7).

Table 7 – Correlation of cortisol levels (μg/L) with anxiety, depression, internalizing and externalizing symptoms, for each subgroup of the sample.

Measures	Boys	Girls	High functioning family	Low functioning family	Obese children
EADS-C Anxiety symptoms	r_s = -0.043 p = 0.832 n = 27	r_s = -0.149 p = 0.519 n = 21	r_s = 0.479 p = 0.071 n = 15	r_s = -0.308 p =0.356 n = 11	r_s = -0.191 p = 0.321 n = 29
EADS-C Depression symptoms	r_s = -0.080 p = 0.692 n = 27	r_s = -0.095 p = 0.683 n = 21	r_s = 0.355 p = 0.194 n = 15	r_s = -0.185 p =0.587 n = 11	r_s = -0.284 p = 0.136 n = 29
EADS Anxiety symptoms	r_s = 0.149 p = 0.442 n = 29	r_s = -0.464 p = 0.026 n = 23	r_s = -0.328 p = 0.102 n = 26	r_s = -0.485 p = 0.093 n = 13	r_s = -0.096 p = 0.628 n = 28
EADS Depression symptoms	r_s = 0.064 p = 0.742 n = 29	r_s = -0.016 p = 0.943 n = 23	r_s = -0.081 p = 0.692 n = 26	r_s = 0.103 p = 0.738 n = 13	r_s = 0.113 p = 0.565 n = 28
CBCL Internalizing symptoms	r_s = 0.001 p = 0.995 n = 27	r_s = 0.121 p = 0.583 n = 23	r_s = 0.331 p = 0.114 n = 24	r_s = -0.019 p = 0.950 n = 13	r_s = -0.080 p = 0.684 n = 28
CBCL Externalizing symptoms	r_s = 0.174 p = 0.386 n = 27	r_s = -0.375 p = 0.078 n = 23	r_s = 0.021 p = 0.924 n = 24	r_s = -0.225 p = 0.460 n = 13	r_s = -0.166 p = 0.399 n = 28

EADS-C: Escala de Ansiedade, depressão e stresse, child version; EADS: Escala de Ansiedade, depressão e stresse, adult version; CBCL: Child Behaviour Checklist; r_s: Spearman correlation coefficient; p: significance level, n: number of children per correlation.

The distribution of symptom scores and cortisol levels according to gender and family functioning is presented in Table 8. In low functioning families, girls scored significantly lower for internalizing problems (CBCL) than boys (Kruskal-Wallis, p=0.018). In high functioning families, both genders scored similarly (Table 8). No significant differences were found when considering BMI categories.

Table 8 - Symptom scores (anxiety, depression and internalizing symptoms) and cortisol levels as median (P_{25}-P_{75}), according to gender and family functioning.

Measures	High functioning family		Low functioning family	
	Boys	**Girls**	**Boys**	**Girls**
EADS Anxiety symptoms	10.0 (2.0-21.8) n=18	7.0 (2.0-17.5) n=17	14.0 (10.0-19.0) n=11	3.5 (1.5-26.8) n=6
EADS Depression symptoms	14.0 (4.5-32.3) n=18	17.0 (2.5-24.0) n=17	14.0 (6.0-21.0) n=11	19.0 (0-44.5) n=6
CBCL Internalizing symptoms	12.0 (9.5-14.5) n=17	11.0 (8.0-16.0) n=15	14.0 (9.0-22.0) n=10	5.0 (2.0-7.5) n=5
Cortisol (µg/dl)	8.5 (6.7-11.7) n=14	10.6 (6.8-13.0) n=13	11.2 (8.8-16.8) n=9	15.2 (5.8-17.5) n=4

EADS: Escala de Ansiedade, depressão e stresse, adult version; CBCL: Child Behaviour Checklist.

Table 9 shows the results for the correlation analyses and significance tests between serum cortisol levels and symptom scores (anxiety, depression, internalizing and externalizing symptoms), for each of the four subgroups (according to gender and family functioning). There was a significant, negative correlation between obese girls' cortisol and their mothers' self-reported anxiety symptoms limited to high functioning families (r_s=-0.779; p=0.003). No association was found between child serum cortisol and maternal self-reported depressive symptoms.

Table 9 – Rank correlation analyses between serum cortisol levels and symptom scores (anxiety, depression, internalizing and externalizing symptoms), for each subgroup (according to gender and family functioning) of the sample.

	High functioning Family		**Low functioning Family**	
	Boys	**Girls**	**Boys**	**Girls**
EADS-C Anxiety symptoms	r_s =0.546 p = 0.205 n = 7	r_s = 0.374 p = 0.362 n = 8	r_s = -0.132 p = 0.778 n = 7	r_s = -0.800 p = 0.200 n = 4
EADS-C Depression symptoms	r_s = 0.577 p = 0.175 n = 7	r_s = 0.195 p = 0.643 n = 8	r_s = -0.019 p = 0.968 n = 7	r_s = -1.000 - n = 4
EADS Anxiety symptoms	r_s = 0.151 p = 0.607 n = 14	r_s = -0.779 p = 0.003 n = 12	r_s = -0.313 p = 0.412 n = 9	r_s = -0.316 p = 0.684 n = 4
EADS Depression symptoms	r_s = 0.144 p = 0.624 n = 14	r_s = -0.293 p = 0.355 n = 12	r_s = -0.076 p = 0.846 n = 9	r_s = 0.738 p = 0.262 n = 4
CBCL Internalizing symptoms	r_s = 0.346 p = 0.247 n = 13	r_s = 0.347 p = 0.296 n = 11	r_s = 0.139 p = 0.721 n = 9	r_s < 0.001 p = 1.000 n = 4
CBCL Externalizing symptoms	r_s = 0.483 p = 0.094 n = 13	r_s = -0.474 p = 0.141 n = 11	r_s = 0.030 p = 0.940 n = 9	r_s = -0.316 p = 0.684 n = 4

EADS-C: Escala de Ansiedade, depressão e stresse, child version; EADS: Escala de Ansiedade, depressão e stresse, adult version; CBCL: Child Behaviour Checklist; r_s: Spearman correlation coefficient; p: significance level, n: number of children per correlation.

Synthesis

This observational study explores potential links between obese children's cortisol, and maternal mental state, family functioning, and the children's symptoms of anxiety and depression.

A non-random sample of 104 obese children (55 boys), mean age 10.9 years (standard deviation 1.76), was recruited from a childhood obesity clinic. Obesity was defined as BMI above the 95th age- and gender-specific percentiles. Neuroendocrine biomarkers were measured. Symptoms of anxiety and depression were assessed with self and parent-reported questionnaires (EADS; CBCL). Family functioning was assessed with parent-reported questionnaires (FACES-III).

A significant, negative correlation (r_s=-0.779; p=0.003) between girls' cortisol and their mothers' anxiety symptoms was found, limited to high functioning families. Boys scored significantly higher than girls on mother-reported internalizing symptoms but not on self-report. No association was found between cortisol in children and maternal depressive symptoms.

CHAPTER VI

STUDY II: ATTACHMENT STRATEGIES AND NEUROENDOCRINE BIOMARKERS IN OBESE CHILDREN

The following publication constitutes part of this chapter:

Pinto I, Wilkinson S, Virella D, Alves M, Calhau C, Coelho R. *Attachment Strategies and Neuroendocrine Biomarkers in Obese Children* Acta Med Port. 2016; 29(5):332-339. [6]

Theoretical Framework

The child-parent relationship plays a major role in the child's early life, influencing socio-emotional development, emotion regulation abilities and leading to development of particular attachment strategies (Bowlby, 1988; Kerns, et al., 2014; Fonagy, et al., 2002; Cassidy, et al., 2013). The quality of the relationship influences both behavioural and metabolic regulation systems as they are activated in stressful contexts (Oskis, et al., 2011). Obese children's attachment strategies have not received much attention. There is evidence that weight is one factor associated with dysregulation in HPA axis activity (Patterson, et al., 2013).

Families shape children's eating habits through feeding and parenting practices (Birch & Ventura, 2009). Stress and maladaptive emotion regulation strategies lead to an increased food intake (Evers, et al., 2010). As adaptive emotion regulation strategies appear to protect from overweight and obesity (Schlam, et al., 2013), the relationship between the main caregiver and the child as a source of healthy emotion regulation has increasingly been considered in studies of obesity development.

A characteristic dysfunctional pattern of interaction is found in families with obese children (Cromley, et al., 2010). Children with difficult temperament and insensitive mothers were found to have significantly higher risk of being overweight or obese during

[6] See Chapter IX, Paper II

school age (Wu, et al., 2011) as well as children in families with mealtime challenges, maternal distress and family conflicts (Zeller, et al., 2007). Parents may overfeed difficult children in a misunderstanding of being good parents and in an effort to calm or reduce the children emotional intensity (Carey, 1985). In this way regulation with food begins in infancy and could continue throughout the childhood.

Methods for evaluating pre-pubertal children's attachment strategies have not been well disseminated or approved within all sections from those working in the development of attachment classification procedures, with the result that there is little information about any relations between metabolic indicators and attachment strategies in this age group. Hypotheses have been developed based on research in younger children (O'Connor, et al., 2007).

Secure attachment (Type B) is associated with the child's approach to his mother at times of need, and is interpreted as a fundamental expectation, a basic trust in the mother's availability and responsiveness. The mother appears to have the child 'in mind' and she is available at times of her child's distress. Through contingent responsiveness, she enables her child to regulate his state, first via her direct handling of the child and subsequently through enabling her child's self-contained management of his affect – an external process facilitates internalizing of the affect regulation. In contrast, the child of a consistently insensitive and unresponsive mother does not learn to expect her mother to be available in stressful situations and develops an insecure-avoidant attachment (Type A). On the other hand, the inconsistently available mother responds to escalating displays of affect in her child, which forces her to respond on the basis of an intermittent reinforcement schedule, but the child remains ambivalent about close contact when proffered as it was only elicited under duress (insecure-resistant attachment, Type C). The Type A child focuses on the environment at the moment of reunion, appearing as if he was ignoring his mother, or appearing to approach her but with objects between them, preventing the distress of potentially not being acknowledged. The reunion behaviour of the Type C child is characterized by anxious contact seeking and clinging, while, at the same time, ambivalently resisting contact with her mother.

It appears that only little or no excess adrenocortical activation is observed in a securely attached child when separated from his mother (Oskis, et al., 2011; Spangler, et al., 1999; Tarullo, et al., 2006). The findings in the insecure-avoidant and insecure-resistant attachment groups are inconsistent. In some studies, both insecure groups were found to

have elevated cortisol levels (Spangler, et al., 1999), others found increased cortisol levels only for insecure-resistant children (Spangler, et al., 2010). These may to some degree reflect disagreements about how to classify particular behavioural strategies - see for example Crittenden & Newman, 2010, which shows how widely different the classification procedures of the DMM and ABCD systems can be. Nevertheless it is theoretically coherent to expect elevated cortisol levels with Type C, as those children are exposed to chronic unpredictable stress, their parents responding to their distress only intermittently. Such findings point to cortisol levels being, at least partly, affected by the caregiving environment (Hutt, et al., 2013; Meaney, et al., 2001).

Evidence of the contribution of caregiving processes to the development and maintenance of obesity has been mixed (Patterson, et al., 2013; Ouellet-Morin, et al., 2008; Francis, et al., 2013). The environmental factors contributing to cortisol reactivity and associated neuroendocrine changes have yet to be documented in people with obesity (Ouellet-Morin, et al., 2008). Differentiating varieties of insecure attachment may help explain variability in cortisol reactivity, since inter-individual variability in HPA-axis activity appears to vary with Type A and C strategies (Gander, et al., 2015; Kiel, et al., 2013). Cortisol, a product of HPA-axis activation, inhibits its own release through negative feedback actions at the level of the pituitary and other brain areas. Previous data indicate that GC can inhibit the HPT axis, at the level of the hypothalamus and pituitary (Santos, et al., 2012). Because both the HPA and HPT axes are stress responsive endocrine systems, it has been of interest to determine if there is any cross-regulation or communication between the HPA and HPT axes.

There are few Portuguese studies correlating cortisol and TSH levels with neuropsychiatric conditions for children of this age. Some independent studies of Portuguese cohorts have been published relating interrelations between the pituitary-thyroid axis and major neurosignaling systems involved in schizophrenia's pathophysiology. The available evidence supports that thyroid hormones deregulation is a common feature in schizophrenia and the fine-tuning of crucial brain networks warrants thyroid hormones homeostasis (Santos, et al., 2012).

Obesity has been associated with changes in HPA and HPT axes activity, attributed to disturbance of the feedback system (Holsboer, et al., 2008). The feedback loop is crucial in recovery from stress, which in turn is essential for healthy physiological and behavioural regulation.

Objectives and Hypothesis

- To examine the quality of attachment in relation to various neuroendocrine biomarkers;
- To investigate these relations for the subtypes of insecure attachment: insecure-avoidant and insecure-resistant attachment strategies;
- Consider these associations in individuals with low versus high family functioning, and in boys versus girls, to gain more insight in the role of family functioning and gender.

We analyzed the hypothesis of an association between the strategies of attachment of obese children and their neuroendocrine biomarkers.

Methods

Sample and Procedure

A convenience sample of children attending the Child Obesity Paediatric Unit of a Tertiary Paediatric Hospital in Greater Lisbon, was drawn from pre-pubertal obese children recruited for this project named "Roots of Early Obesity" (Pinto, et al., 2011). Participants were those for whom detailed anthropometric information and behavioural measurements of the child's development were feasible to collect. Exclusion criteria were the use of medication, having already reached puberty during the period of assessment, undergoing mental health intervention or other medical treatment besides those for obesity.

Parental written, informed consent was obtained. The project was approved by the Hospital Medical Ethics Committee.

Measures

Attachment Strategies

The attachment strategies of children were assessed using "IVIA - Inventário sobre a Vinculação na Infância e Adolescência" (IACA – Inventory of Attachment in Childhood and Adolescence), a parent-reported questionnaire originally developed and validated in Portugal (Soares, et al., 2007). IVIA was designed to classify child-caregiver attachment strategies in childhood and adolescence, from 7 to 17 years of age. It categorizes attachment strategies as secure (Type B), insecure-avoidant (Type A), or insecure-resistant

(Type C). IVIA does not consider Type D, insecure-disorganized/disoriented strategy (Main, et al., 1986), following instead the Dynamic Maturational Model of attachment and adaptation (DMM) classificatory system (Crittenden, 2006; Kozlowska, et al., 2011). Satisfactory psychometric properties were reported (Soares, et al., 2007).

Family Functioning

Family functioning and typology were assessed using FACES III (Family Adaptation and Cohesion Scale) (Olson, 1985), a parent-reported questionnaire, validated for Portugal (Curral, et al., 1999). Only one parent per child was surveyed, most often the mother. This self-reported tool is a 20-item Lykert type questionnaire that evaluates 2 major dimensions on the circumplex model: Cohesion and Family Adaptation. Reliability for the cohesion is 0.78 and for the adaptability is 0.70 (Curral, et al., 1999).

This tool assesses the degree to which family members are adaptive and connected to each other. The circumplex model is a classification system with 16 family types, organized in three general types: balanced, mid-range, and extreme. The family adaptability is classified in four levels: rigid, structured, flexible, and chaotic. The two central levels (structured and flexible) are considered as balanced family adaptability and the two extreme levels (rigid and chaotic) are considered as unbalanced family adaptability. The family cohesion is classified in four levels: disengaged, separated, connected, and enmeshed. The two central levels (separated and connected) are considered as balanced family cohesion and the two extreme levels (disengaged and enmeshed) are considered as unbalanced family cohesion (Olson, 1985). In other words, balanced levels of cohesion and adaptability are conceptualised as reflecting healthier family functioning, and they are classified as "high functioning families". Unbalanced levels of cohesion and adaptability (very low or very high levels) are seen as reflecting more problematic family functioning, and they are classified as "low functioning families".

Metabolic Assessment

Height, weight, waist circumference, blood pressure and heart rate were measured following standardized protocols. BMI ($kg.m^{-2}$) was calculated using height and weight. Obesity was defined as BMI $\geq 95^{th}$ age and gender-specific percentile (Barlow, et al., 2007). Blood pressure was measured three times and the third reading was recorded (O'Brien, et al., 2004). Age and gender-specific percentiles were used (O'Brien, et al., 2004).

As part of the "Roots of Early Obesity" project, fasting blood samples were drawn at 8 a.m through an indwelling i.v. catheter, after 45 min of rest. Children were considered healthy and had not taken any medication. Plasma cortisol, glucose, insulin, HDL and LDL cholesterol, triglycerides, catecholamines, TSH and T4 were measured using the standard procedures of the Clinical Pathology Laboratory of the institution. TSH ≥ 3.1 μIU/mL was considered as laboratorial evidence of hypothyroidism.

Statistical Analyses

The patient characteristics were described using the mean and standard deviation (SD) or median and interquartile range (IQR: 25th percentile-75th percentile) for continuous variables, as well as frequencies (percentages) for categorical variables. To explore the association between attachment strategies (modeled as nominal variables), age, gender, family functioning and neuroendocrine and metabolic assessment measures, univariable linear regression models were applied.

Strategies Types A and C were combined to provide an "insecure attachment" group (a standard practice using the Berkeley system for attachment classification associated with Mary Main), for multivariable analysis. Variables that had a significant association with each attachment strategy were included in multivariable explanatory models tested by multiple linear regression, in which gender, BMI percentile and the classification of family functioning were always included. A level of $\alpha = 0.05$ was considered to be significant. Statistical analysis was carried out using Statistical Package for the Social Science for Windows version 22.0 (SPSS Inc, Chicago IL).

Results

From 83 recruited pre-pubertal children, 73 children (40 boys) with all the required data were included. The sample had a mean age 10.86 (SD = 1.8) years. The BMI was above the 97th percentile in 68 children (93%).

The distribution of the measurements of the neuroendocrine values and metabolic indicators is presented in Table 10.

Table 10 - Descriptives of the neuroendocrine and metabolic indicators.

Parameter (n)	Mean (SD)	Median	Min.-Max.	25th P-75th P
Glucose (mg/dl) (53)	93.96 (9.356)	93.0	71 – 120	89.0 - 98.0
Insulin (U/ml) (39)	14.45 (9.85)	13.3	0.28 - 52.95	7.99 - 16.50
Triglycerides (mg/dl) (48)	97.5 (39.85)	89.0	33 – 187	68.0 - 129.75
Cholesterol (mg/dl) (50)	162.7 (31.52)	166.0	49 – 227	145.0 - 184.5
HDL (mg/dl) (47)	48.6 (14.24)	45.0	24 – 91	38.0 - 57.0
LDL (mg/dl) (45)	108.7 (23.78)	110.0	59 – 167	96.5 - 120.0
ALT (U/L) (46)	27.35 (23.86)	22.5	11 – 167	16.75 - 30.0
AST (U/L) (44)	23.75 (7.59)	23.5	12 – 56	18.25 - 26.0
TSH (µIU/ml) (52)	2.8 (1.21)	2.68	0.72 - 5.92	1.7 - 3.5
T4 (ng/dl) (49)	1.3 (2.19)	1.0	0.59 - 16.20	0.8 - 1.2
ACTH (pg/ml) (49)	26.4 (16.94)	20.65	5.35 – 105.0	16.6 – 33.2
Cortisol(µg/dl) (53)	10.7 (5.58)	8.8	3.3 - 29.0	6.7 - 13.5

Glucose - Basal Plasma Glucose; Max – maximum; Min.- minimum; n – number of assessed children; P – percentile; SD – standard deviation.

From the univariable analysis between attachment strategies and the neuroendocrine and metabolic indicators, ACTH, cortisol, TSH and hypothyroidism were identified as potentially associated with Secure Attachment (Type B), "Insecure Attachment" (Types A, C and D in the Berkeley system) (Table 11A) and Avoidant Attachment (Type A) (Table 11B).

Table 11A - Associations between attachment strategies (Secure and Insecure) and anthropometric, familiar and neuroendocrine variables (univariable analysis).

	Secure Attachment (Type B)		**"Insecure Attachment" (Types A, C and D in the Berkeley System)**	
	β-estimate (95% CI)	**p-value**	**β-estimate (95% CI)**	**p-value**
Gender (boys)	-0.007 (-0.095;0.082)	0.882	0.072 (-0.069;0.213)	0.309
BMI percentile	0.035 (-0.168;0.237)	0.731	0.119 (-0.200;0.439)	0.457
Family Functioning (high level)	0.027 (-0.078;0.131)	0.610	-0.012 (-0.179;0.154)	0.883
Hypothyroidism	0.063 (-0.053;0.180)	0.277	-0.001 (-0.186;0.184)	0.989
TSH (µIU/ml)	-0.001 (-0.051;0.049)	0.962	0.069 (-0.006;0.144)	0.072
ACTH (pg/ml)	-0.003 (-0.005;0.000)	0.061	-0.005 (-0.010;-0.001)	0.030
Cortisol (µg/dl)	0.002 (-0.008;0.012)	0.672	-0.016 (-0.030;-0.002)	0.026

ACTH – adrenocorticotropic hormone; BMI – body mass index; CI – confidence interval; TSH - thyroid-stimulating hormone.

Table 11B - Associations between attachment strategies (Avoidant and Resistant) and anthropometric, familiar and neuroendocrine variables (univariable analysis).

	Avoidant Attachment (Type A)		Resistant Attachment (Type C)	
	β-estimate (95% CI)	p-value	β-estimate (95% CI)	p-value
Gender (boys)	0.056 (-0.028;0.140)	0.187	0.016 (-0.075;0.107)	0.724
BMI percentile	0.141 (-0.050;0.332)	0.144	-0.021 (-0.226;0.183)	0.834
Family Functioning (high level)	-0.051 (-0.153;0.051)	0.316	0.039 (-0.066;0.143)	0.456
Hypothyroidism	-0.012 (-0.122;0.097)	0.820	0.011 (-0.112;0.134)	0.855
TSH (μIU/ml)	0.048 (0.004;0.091)	0.034	0.021 (-0.030;0.073)	0.405
ACTH (pg/ml)	-0.003 (-0.006;0.000)	0.032	-0.021 (-0.005;0.001)	0.142
Cortisol (μg/dl)	-0.008 (-0.016;0.001)	0.075	-0.008 (-0.017;0.001)	0.069

ACTH – adrenocorticotropic hormone; BMI – body mass index; CI – confidence interval; TSH - thyroid-stimulating hormone.

The main results of multivariable analysis of the explanatory models for the attachment strategies are shown in Table 12. Type A, Avoidant attachment strategies, had significant positive association with TSH levels and negative association with cortisol levels (R^2 = 0.352). Type B, Secure attachment strategies, had significant positive associations with both hypothyroidism and BMI percentile (R^2 = 0.541). The "Insecure attachment"

strategies group showed some evidence of positive association with TSH (R^2 = 0.250). ACTH was not found correlated with any type of attachment. No explanatory model could be derived for Type C, Resistant attachment strategies.

Table 12 - Adjusted association between attachment strategies and anthropometric, familiar and neuroendocrine variables (multiple linear regression analyses). No associations with Resistant Attachment (Type C) were found.

	Associated variables	β-estimate (95% CI)	p-value	R^2
Avoidant Attachment (Type A)	Cortisol	-0.015 (-0.028;-0.001)	0.036	0.352
	TSH	0.084 (0.015;0.154)	0.021	
Secure Attachment (Type B)	Hypothyroidism	0.207 (0.092;0.321)	0.002	0.541
	BMI percentile	0.291 (0.104;0.477)	0.004	
"Insecure Attachment"	TSH	0.128 (0.000;0.256)	0.050	0.250
	ACTH	-0.011 (-0.025;0.002)	0.087	

Variables in the models: gender, BMI percentile, family functioning classification, hypothyroidism, cortisol, ACTH, TSH.(ACTH – adrenocorticotropic hormone; BMI – body mass index; CI – confidence interval; TSH - thyroid-stimulating hormone).

Synthesis

Quality of the parent-child relationship influences the mechanisms of development of the child's physiological stress regulation. This study explored associations between attachment strategies and both cortisol and TSH, hypothesized to be respectively a potential mediator and a potential intervening variable of the parent-child relationship in obese children.

A sample of 83 obese children (46 boys), aged 10.9 (1.8) years was recruited from a child obesity clinic. Obesity was defined by BMI percentile adjusted for age and sex. Metabolic biomarkers were measured by routine methods. Attachment strategies were assessed with parent-report questionnaires (IACA). Family functioning was assessed with parent-reported questionnaires (FACES-III). Multivariate linear regression analyses were performed.

Type A, avoidant attachment strategies, had significant positive association with TSH levels and negative association with cortisol levels (R^2 = 0.352). Type B, secure attachment strategies, had significant positive associations with both hypothyroidism and BMI percentile (R^2 = 0.541). "Insecure attachment" (Types A and C combined) strategies showed some evidence of positive association with TSH (R^2 = 0.250).

CHAPTER VII

DISCUSSION

The following publications[7] constitute part of this chapter:

Pinto I, Wilkinson S, Virella D, Alves M, Calhau C, Coelho R. *Anxiety, family functioning and neuroendocrine biomarkers in obese children.* Acta Med Port. 2017; 30(4): 273-280.

Pinto I, Wilkinson S, Virella D, Alves M, Calhau C, Coelho R. *Attachment Strategies and Neuroendocrine Biomarkers in Obese Children.* Acta Med Port. 2016; 29(5):332-339.

Main Findings and their Clinical Relevance

In this chapter, the most important results will be presented and discussed in relation to the previous findings. In chapter VIII the results of this evaluation will be used to formulate their potential application in reducing obesity in a population of pre-pubertal children.

Study I:

Anxiety, Family Functioning and Neuroendocrine Biomarkers in Obese Children

This study explored associations between basal serum cortisol levels and symptoms of anxiety and depression in a sample of obese children. We found a significant negative association between serum cortisol and the mothers' anxiety symptoms, limited to girls and particularly significant for girls from apparently high functioning families. A significant, positive correlation between symptoms of anxiety and depression in children and mothers was found. There was a significant, strong positive correlation between mothers' and daughters' symptoms of depression. Boys scored significantly higher for parent-reported internalizing symptoms than girls. Mothers of obese boys scored markedly higher for symptoms of depression than mothers of obese girls. No associations were found between the children's serum cortisol and their mothers' depressive symptoms, indicating that the association that was found between serum cortisol and mothers' anxiety symptoms was specific for anxiety.

[7] See Chapter IX

One possible explanation for this association between mothers' anxiety symptoms and children's cortisol levels is that it is secondary to alterations in HPA-system activity present from birth. HPA-system functioning is at least partially determined by genetic (Dedovic, et al., 2015; Pasquali, et al., 2012) and prenatal factors (Radtke, et al., 2015). For example, CRH gene regulation involves multiple activating and repressing transcription factors, specifically the GR and cyclic adenosine monophosphate (cAMP). Another important biological aspect is timing in the transcription of the signals, which is critical for effective GC repression of the cAMP induced CRH gene (Pasquali, et al., 2012). Therefore, differences in timing of stimulatory and repression signals may be important for adaptation to stress, thereby providing a molecular explanation for the variability in adaptation to stress. These factors may influence CRH secretion patterns (Coleman, et al., 2014), feedback effects of cortisol on central GR, or both (Pasquali, et al., 2012).

On theoretical grounds, one can expect alterations in HPA-system activity to be more evident in individuals with a long history of anxiety symptoms. These changes may be reasonably interpreted as expressing a state of accrued vulnerability which is akin to some increment in maternal stress, in particular during the latter half of pregnancy. Such undesirable conditions are known to favour maternal weight gain during pregnancy (Lillycrop, et al., 2011). An overactive HPA-system leading to elevated cortisol levels is associated with an increased prevalence of abdominal obesity, although previous studies have suggested that there are obese individuals who may have desensitization to GC, thereby not exhibiting increased blood cortisol (Pasquali, et al., 2012). A well accepted hypothesis is that once the HPA-system is over-activated during early development, it remains permanently unstable, vulnerable or dysfunctional, possibly due to transcriptional/epigenomic mechanisms (Lajud, et al., 2015).

These elevations could influence down-regulation of components of the HPA-system, as suggested by the Adaptive Calibration Model (Del Giudice, et al., 2011), an evolutionary-developmental theory of individual differences in the functioning of the stress response system. HPA activity is regulated by a hierarchy of feedback loops at different levels in the axis, and the sensitivity of these feedback loops is a major factor in determining HPA responsivity. The HPA-system responds to chronic stressors with sustained cortisol elevation, resulting in a flattened diurnal rhythm of secretion. Chronic elevation is often followed by a negative rebound of the system to below the previous baseline level after the stressor terminates. This hypocortisolism phase can last months; its function is probably to facilitate recovery and offset the physiological and immune costs of high circulating cortisol

(Del Giudice, et al., 2011). Hence, high and persistent levels of anxiety could be associated with low cortisol concentrations, reflecting resilience rather than a risk for psychopathology (Planalp, et al., 2015).

This is in accordance with the present study´s findings for girls. We found a significant negative association between serum cortisol and mothers' anxiety symptoms, limited to girls, and particularly significant for girls from apparently high functioning families.

Family functioning might operate through either a threshold effect, or be secondary to reduced buffering effects of protective relationships (Sabin, et al., 2015). Possibly mothers with high levels of anxiety symptoms influence their children's vulnerability to anxiety symptoms through an excess of control. In fact, some overprotective parenting behaviour has been associated with increased risk for anxiety symptoms in children (Waite, et al., 2015; Dallman, et al., 2010). It is also proposed that affects are "infectious" – and mirror neurone influences may be at play so that the affective state of the parent is contagious. We already know that successful treatment of a depressive disorder in adolescents depends on treating any concomitant parental depressive disorder at the same time. But we are unaware of studies having looked at the same for anxiety.

In the group from low functioning families, the association between mothers' anxiety symptoms and cortisol was not found to be statistically significant. However, this limitation may well be just a reflection of the small sample sizes of children for whom complete data-sets were obtained (Table 9).

There is another possibility to explain the slightly paradoxical finding of a relationship between higher functioning, maternal anxiety symptoms and girls' cortisol. The findings depend on self-reports from the mothers, and it may be that mothers from lower functioning families, particularly those high on rigidity and disengaged, are less clear about reporting on their subjective affective states. The relation between family functioning and quality of the self-reported information needs further investigation. Nevertheless, there is a definite relation between maternal mental state and biomarkers in their children. Future research needs to look at whether there is a connection between maternal mental state, maternal awareness of their children's diverse forms of distress, including those of anxiety and depression, and family functioning. Research into these factors could then be linked to research on the role played by maternal and child attachment strategies. The finding about lower reporting of internalizing symptoms in girls from low functioning families, may also

be a result of lower maternal awareness of their daughters' subjective discomforts, rather than an absolute finding that these girls had actually less internalizing distress. To create a bridge to attachment theory, we would then be proposing that these girls had developed Type A attachment strategies to enable their self-sufficiency in the light of minimal maternal sensitivity to their distress. Mothers of obese boys scored markedly higher for symptoms of depression than mothers of obese girls.

Several community studies of children have assessed both depression and anxiety (Velez, et al., 1989). Results from these studies show that anxiety is highly prevalent among depressed children, with an estimated prevalence ranging from 38% (Costello, et al., 1988) to 72% (Anderson, et al., 1987). This is coherent with the present study's finding of a significant, positive correlation between symptoms of anxiety and depression in children, stronger in girls. Although anxiety is highly comorbid among depressed children, the age at which the onset of depression and anxiety occur relative to one another has not been explored systematically. The study by Kovacs and colleagues (Kovacs, et al., 1989) has shown that anxiety precedes the onset of depression in 67% of the comorbid cases. This finding suggests a developmental sequence in the relationship between the two disorders, with later comorbid depression and anxiety having emerged first as anxiety.

We found a significant, positive correlation between maternal symptoms of anxiety and depression, stronger in the girls' mothers. Evidence from studies of depressed adults suggests that depression is often preceded by anxiety and that is more likely to be true among adults whose depression began before age 25 (Parker, et al., 1997). The results of our study are coherent with the research that for females there is 100% certainty that if in adolescence they were either anxious or depressed, by the time they were 25 years they have also been depressed – whereas for males it is only 80% (Parker, et al., 1997).

In the present study, symptoms of depression in mothers had a significant, positive correlation with children's symptoms of depression, stronger in girls. No associations were found with maternal symptoms of anxiety. A depressed and withdrawn mother may elicit in her child a Type A strategy such as compulsive caregiving or compliance (Hautamäki, et al., 2010). When these strategies fail, the child may withdraw and/or show signs of depression (an awareness that nothing he can do will elicit anything more than rudimentary physical care). Thus, if the parents feel threatened, and they lack the possibility to change their situation, their self-protective strategies may create a threat to their child, who has to organize his attachment strategy around this indirect threat. Depressed mothers respond to

their girls and boys differently. There is a reduced amount of talk with their boys, but not with their girls, and they are much more critical of their sons compared to their daughters. The effects depend a bit on age at which the child was exposed to the depressed mother. This is related to early parenting, but in the present study it was only measured at the time of the study. In the present study, the positive correlation between mother's symptoms of depression and children's symptoms of anxiety and depression, confirms that it isn't to be expected that it will be possible to treat successfully a depressed child unless the parent's depression is also treated. Marital issues will need to be included.

Studies have provided consistent evidence that the children of depressed mothers are themselves at increased risk for developing depression (Nomura, et al., 2002). This possibly reflects a genetic or familial component of susceptibility to depression, alterations to the fetal environment (such as more cortisol crossing the placental barrier in depressed mothers), and in the postnatal period, deviations from appropriate and effective child-rearing by depressed parents that interfere with children's healthy emotional development. All of these mechanisms could also affect children's HPA regulation. Should the children of depressed mothers demonstrate adrenocortical dysregulation prior to evidencing their own depressive symptoms, this could be taken as further evidence that atypical HPA axis activity is a precursor of, and possibly contributing factor to, pediatric depression. Girls appear to show dysfunctional physiological reactivity to interpersonal stressors that might increase their risk for developing affective related problems. These findings of gender differences in the links with HPA axis functioning echo other literature that has hypothesized that HPA dysregulation might function as one mechanism underlying sex differences in depression (Stroud, et al., 2009).

Considered together, these results contribute to the recent literature about maternal affective problems being associated with atypical HPA axis functioning in their children (Halligan, et al., 2007). This is evidenced across developmental periods and principally in disruptions to basal cortisol levels. The children's altered cortisol levels are apparently prior to there being evidence that they are experiencing mental health problems. Results from research assessing basal HPA axis functioning and diurnal variation among child and adolescent samples show some continuity with corresponding work in adults.

Children's BMI was not significantly associated with internalizing or externalizing symptoms. Elevated levels of behavioural and emotional difficulties as well as peer problems or social withdrawal have been reported for obese compared to non-obese

children and adolescents, particularly in treatment-seeking samples (e.g., Vila, et al., 2004), whereas in non-clinical samples these associations were less clear (Puder & Munsch, 2010). As our sample consisted of non-clinical participants, because an exclusion criteria for the study was ongoing mental health problems, this might explain our non-significant findings. Furthermore, the children of our sample might have been too young to have already developed fixed psychosocial patterns associated with obesity development in childhood. Goodyer has suggested that changes at adrenarche are essential for setting vulnerability to depression (Goodyer, et al., 2001). Parental reports on children at this young age may also not accurately capture internalizing symptoms.

On the other hand, investigating the role of genetic, epigenetic, attachment strategies and their interactions was beyond the scope of the present study. The role of attachment strategies is addressed in Study II (Pinto, et al., 2016).

Study II:
Attachment Strategies and Neuroendocrine Biomarkers in Obese Children

Associations between neuro-endocrine and metabolic indicators and attachment strategies were explored in a convenience sample of pre-pubertal obese children from a child obesity clinic. Detailed somatic and behavioural measurements of the child's development and family functioning were collected.

Using multivariable analysis, we found evidence of the association between avoidant attachment strategies (Type A) with higher TSH levels and lower cortisol levels. On the other hand, secure attachment strategies (Type B) were associated with both the presence of laboratorial evidence of hypothyroidism and higher BMI percentile.

We investigated the role of, and interactions between, several biological and environmental factors to see if any specific sub-groups could be identified which may benefit for further description and specific treatment strategies. Type A strategies were found to exhibit a negative, strong association with cortisol levels (β-estimate=-0.015; p=0.036; R^2 = 0.352). This finding offers evidence of an increased risk for dysfunctional HPA-axis in children that score high in Type A attachment strategies.

As previously reported (Spangler, et al., 2010), insecure attached infants (classified after Berkeley ABCD system) have been found to display high cortisol levels after a stressful stimulus. This possibility remains and it seems to be the result of different classification procedures, notwithstanding the fact that this finding was not independently

validated by other researchers (Doom, et al., 2013; Goldberg, et al., 2003). The high activation of the HPA system of insecure children may not terminate soon after their reunion with their primary caregivers, because children are unable to use effectively the attachment figure for their regulation, making it difficult for a state of homeostasis to be reached (Gander, et al., 2015).

Von der Lippe and Crittenden (2000) argue that the child organizes his attachment behaviour in ways that increase the probability of his parents providing protection from dangers and comfort or decrease the probability of parents rejecting or harming the child. Individuals using a Type A strategy inhibit affect also under threat and may even produce compulsive behaviour, role-reversing and obedient behaviour. Schore (1994) argues, using Bowlby's (1969/1988) classical model on sequential responses to physical separations that the child may gradually shift from a sympathetic-dominant distress state, *protest*, towards a parasympathetic dominant state, *despair*. Gradually, the caregiver's lacking ability to respond and to regulate may lead to a relative deactivation of the child's attachment system by excluding from processing socio-emotional stimuli that activate attachment behaviour (Schore, 1994) or in Crittenden terms (2016), excluding negative affect that is, fear, anger and bids for comfort. A system to cope with heightened levels of sympathetic arousal and stimulation may not fully develop, and be coupled to risk of developmental psychopathologies (Schore, 1994; Crittenden, 2016).

Other explanation for these findings is that cortical suppression could be due to a pattern of stress habituation over time, a pattern that increases the risk of difficulties in emotional and behavioural regulation; equally, reduced stress responsiveness may emerge as a result of genetic factors, or gene-environment (GxE) interactions (Van Goozen, et al., 2008). These differing patterns may in part reflect adaptations of the HPA axis to different periods of onset and chronicity, and differential genetic susceptibility. Confounders, such as depression, which is a frequently observed comorbidity, may account for some of the reported differences (Newport, et al., 2004). Investigation of the role of depressive symptoms was beyond the scope of this study. Study I (Pinto, et al., 2017) explored anxiety and depressive symptomatology and other comorbidities in a larger sample.

It seems that childhood maltreatment may lead to atypical responsiveness of the HPA axis to stress, which in turn predisposes to psychiatric vulnerability in later life (Van Goozen, et al., 2008). While there is general agreement around this broad principle, the putative mechanisms of how dysregulation of the HPA axis might mediate the link

between stress and psychopathology and the precise nature of any interaction remains less clear (Newport, et al., 2004). It is possible that diminished cortisol responsiveness may emerge if early chronic stress leads to an initial hyper-activation of the HPA system which then progresses over time to a state of hyporeactivity, as a form of adaptation following sustained exposure to raised levels of ACTH (Herman, et al., 2013).

A pattern of hypoarousal (low cortisol, flat diurnal rhythms) has been termed a low biological sensitivity to context (BSC), (Boyce & Ellis, 2005) and could be linked with developmental processes associated with Type A attachment strategies. A low BSC can be expected to disadvantage the developing child, potentially rendering them less able to cognitively process environmental opportunities and threats, with poorer attention, and less efficient priming for memory storage for emotionally and socially relevant events (Flinn, 2006). Hypoarousal is expected to be associated with mental health symptoms (Gunnar & Vazquez, 2001); indeed, low cortisol has been directly linked with emotion and social regulation (Stetler & Miller, 2005).

The findings in this study suggest that there may be commonalities in the regulation of HPA- and HPT-axes and/or communication between the axes. A common factor, such as developmental processes associated with development of the Type A attachment strategies, may be important in a linkage between the two axes. Furthermore, the correlation between the HPA- and HPT-axes may indicate that activity of one axis alters the activity of the other axis. As mentioned above, previous work has demonstrated that GC, the end product of HPA axis activation, can inhibit the HPT axis (Lobotková, et al., 2014). This study supports this proposition, i.e., that lower cortisol may be associated with higher TSH levels. The meaning of this for developing differentiated treatment approaches remains to be explored.

The fact that we didn't find an association between insecure attachment (Types A, C and D in the Berkeley system) and BMI is in contrast to studies reporting that insecure attachment (Types A, C and D in the Berkeley system) increased the risk for childhood overweight or obesity (Rhee, et al., 2006; Anderson & Whitaker, 2011; Anderson, et al., 2012). The problem seems to be that in the Berkeley system the contrasting Type A and C strategies are lumped together, with Type D, as "insecure", whereas I believe the distinction between Type A and C processes needs to be retained – and would be expected to bring greater clarity to this research field. The way of interpreting the attachment behaviours can be different in different cultures. This will lead to children later developing

different attachment strategies as they adapt the most effective behaviours within the particular semiotic culture (Crittenden & Claussen, 2000). Kocken, *et al.*, found ethnic differences between the parental beliefs of prevention and management of their children' overweight to be essential. Serving and receiving food provides an important context for the development of attachment strategies between the giver and the receiver in the family context. In this way relationship is tied to food and it is important in families. It is easy for mothers to feed and over-feed children, since feeding is an extension of the infancy relation with breastfeeding. In addition, mothers could be sensitive but have difficult children who are especially sensitive themselves or temperamental and therefore specialy difficult to down regulate *e.g.* regarding food.

It is well known and accepted that the negative feedback system is essential in recovery from stressful situations and that a balanced stress recovery system that promotes homeostasis is of great importance. Presumably, an insecure attachment relationship does not facilitate adequate termination of the stress reaction. Now, this lack of homeostasis could put the child at metabolic and developmental risk, as long term negative outcomes have been shown to result from both insecure attachment (Hillman, et al., 2012; Gungor, et al., 2014; Dockray, et al., 2009) and childhood obesity.

Research strengths and limitations

Our goal was to contribute to understanding the complexity of a wide range of associations with cortisol levels in obese children. The study was not designed to test hypotheses, include all possible relevant parameters or be designed to generalize the findings to other samples. Yet, when investigating certain risk factors for a problem that is known to be influenced by several other factors as well, it might not be possible to generalize the findings. Through identifying discrete subsamples it may be possible to identify factors that only have effects under limited circumstances. But our study size means that such subsamples were of necessity very small.

Our study had several strengths. First, we investigated a sample of children during childhood, a time before tendencies toward obesity and behaviour problems become chronic patterns.

This study had also the strength of obtaining both biological and psychometric measures, using validated instruments on a homogeneous sample of obese, pre-pubertal children. The study of the relationship between attachment and psychopathology in clinical

samples of children with emotional and behavioural disorders may lead to results that support the clinical use of IVIA. Indirectly the clear demarcation of the metabolic profile and attachment strategy is a validation of this Portuguese instrument for assessing attachment, but the study should be repeated using other classificatory systems of attachment in this age group. It is likely that children's relationships with adults other than the parents such as with grandparents, teachers or child care providers, also influence the development of children's attachment strategies. However, these other relationships were not assessed in this study.

We directly assessed anthropometric data of children in the laboratory, rather than relying on self-reported data. In comparison with direct measures of anthropometric data, self-reported weight and BMI usually tend to be underestimated, whereas height tends to be overestimated (Gorber, et al., 2007), especially in case of overweight or obesity (Ciarapica, et al., 2010). Hence, our results exclude this reporting bias.

The small number of children affects mostly the power to identify significant associations with biological and psychometric variables alike. The significant associations found in this sample should be considered as reliable because the small discriminant coefficients (R^2) point out the multifactorial nature of the attachment strategies, anxiety and depression symptoms. Some of the potential etiological factors have not been investigated and others were not found significant, potentially due to the sample size. It is impossible to determine the direction of the bias (if any) due to analyzing only those children for whom it was possible to collect all relevant information. It should be noted that the reported associations are merely correlational, and that the mechanisms underlying them need elaboration. Future research must include the non-obese comparison group. In obese infants, these associations remain uncharted (Bar-Haim, et al., 2007).

In general, population measures of HPA reactivity probably are better indicators than measures of baseline HPA activity. In this study, HPA-system activity was not directly assessed in response to a stressor given the ethical and practical implications of pharmacological challenge tests with children. The results do not reflect absolute changes in cortisol levels but shifts in the diurnal rhythm which itself might not be constant when there is anxiety or depression. As ACTH was not found altered in the explored models, cortisol changes may reflect differences in perceived acute stress which were not considered in the present study. Thus, future studies that assess both measures simultaneously may reveal more differentiated results. An ethical dilemma arises with

exposing children to prospective stress, and it may be that one would have to be limited to such situations as arise with starting at a new school or similar.

The findings depend on self-reports primarily from the mothers, rather than both parents, and it may be that mothers from lower functioning families are less clear about reporting on their subjective affective states, for example if their lower functioning was associated with Type A attachment strategies in the mothers. The FACES dimensions suggest different forms of low functioning, *e.g.* low in quality, but including dimensionally opposite extremes. The study cannot elaborate on the potential importance of these different poles of functioning. Within the attachment paradigm we would expect the outliers to be a mixture of those who overemphasise their discomforts for what they can achieve by doing so (Type C), and those who have always displayed little of their affective states, and been poorly aware of what they might be (Type A). The relation between family functioning and quality of the self-reported information underlines the importance of using multiple sources providing both self-report and observations, also while investigating neuroendocrine biomarkers of anxiety and depression (Wilkinson, 2003). Future research examining the specific qualities of lower functioning families will be valuable for the development of prevention or intervention efforts with high-risk children.

CHAPTER VIII

FROM FINDINGS TO THEIR POTENTIAL APPLICATION IN REDUCING OBESITY IN A YOUNG POPULATION

The findings of this thesis point towards some important potential applications which can prove to be of clinical importance. Although our results showed the effect sizes to be small they may prove useful at the population level.

Our findings are coherent with other work, showing that maternal mental state and the developmental processes of the Type A attachment strategy appear to be associated with effects on children' stress regulatory systems.

These results have biopsychosocial plausibility. There is evidence that obesity and the metabolic syndrome can result from physiologic and behavioural response patterns to psychological stress. The physiological mechanisms appear to be related to neuroendocrine pathways such as those involving cortisol. The behavioural mechanisms may include impaired sleep or eating as part of the response pattern to cope with negative emotions, such as fear, sadness, anxiety, and anger. Empirical observations support the possibility that children with secure attachment strategies (Type B) are more easily comforted in stressful situations and are better able to regulate negative emotions; these behaviours are reflected in healthier patterns of physiological responses to stress. Secure attachment could reduce the risk for childhood obesity by preventing frequent or exaggerated stress responses from disrupting the normal functioning and development of physiological systems that affect energy balance, body weight, and fat distribution. Securely attached children who are better able to regulate their emotions can be expected to be less likely to eat in response to emotional distress in early childhood when the systems in the limbic brain that regulate both emotion and appetite are developing concurrently.

Obesity is a complex condition, and the high prevalence seen in children is the result of multiple causes. Although solutions to the public health problem of obesity must take many forms, successful approaches to the prevention of obesity in early childhood are still lacking. As the brain is capable of profound plasticity during childhood and adolescence, these developmental periods might be suitable for interventions, and might

contain opportunities to extenuate the negative consequences of early obesity with associated mental disorders.

If the findings of this thesis are confirmed in other studies, it may be possible to develop obesity prevention strategies that include enabling the individual to generate and apply self-protective strategies at the right time and in the right context. The results of this study are important in providing support for changing the biology of vulnerable children via interventions addressing qualities in their caregiving relationships.

Parents play a key role. The development of children's stress responses depends on how parents protect their children from extreme levels of stress, and respond supportively and consistently to normal levels of stress. Additionally they model behavioural responses to stress and we expect facilitate mirror-neurone development of stress responses. Whether the focus should be counseling, parent education, parenting interventions, or psychotherapy for the adult(s) is the more important question. If the parent is relatively balanced and able to integrate with good enough reflective functioning, counseling might be a good way to enable them to use more effectively skills and information that they already have. If the parent, however, uses a relatively extreme Type A or C strategy and they also display in their daily lives the effects of past exposure to danger (often without being aware that such is the case), it is likely that individual psychotherapy (or marital therapy), should precede or co-occur with parenting work.

It is hypothesized that when negative emotional states are activated, a shift towards lower levels of cognition and self-awareness is initiated. This mechanism tends to remove the inhibitions, thereby facilitating the start of binge eating or overeating, both in clinical (Engelberg, Steiger, Gauvin, Wonderlich, 2007) and in non-clinical subjects (Lyubomirsky, Casper, Sousa, 2001). Thus secure and aware children would be able to read their body signals, both implicit and explicit, and use the signals more appropriately to achieve a greater state of well-being, harnessing their needs. Children whose stress response is well regulated may be less likely, for example, to overeat in response to emotional distress, and may have longer sleep duration, which could also reduce their risk for obesity.

Subtyping insecure attachment may help explain variability in cortisol reactivity, since inter-individual variability in HPA-axis activity appears to vary with Type A and C strategies as was shown in the present study. In DMM, Type A and C are psychologically complementary. The transformations that lead to Type A are based on a different characteristic of the incoming signal and are processed through different parts of the brain,

than are the transformations associated with Type C. Because they result from contrasting processes, they are likely to respond to different approaches. A Type A individual might benefit from techniques that focused on feeling and somatic representation of feeling, whereas this treatment might increase somatic symptoms of stress in a Type C individual. Similarly, a Type C individual might benefit from a behavioural approach emphasizing self-relevant contingencies, whereas this might expand the repertoire of compulsive behaviour of a Type A person.

HPA dysregulation in children tends to be associated with maternal affective problems. Given the gender differences in the rates of depression that also emerge in the context of the transition from childhood to adolescence, it will be essential to further examine gender differences in stress system functioning. It is reasonable to hypothesize that patterns of dysfunctional HPA axis functioning are more pronounced and/or have qualitatively different psychosocial triggers for girls compared to those for boys (e.g., Stroud et al., 2009). This notion is supported by the emerging empirical work indicating that girls with a history of depression exhibit dysfunctional HPA axis activity (Goodman et al., 2011). Although this research requires replication, these findings complement earlier work (e.g., Hankin et al., 2007).

It is not clear whether this pattern of lower cortisol levels is a risk marker for subsequent physical and mental disorders, or is a reflection of individual differences in stress reactivity that may have protected the developing brain from adverse impacts of maltreatment. Both possibilities exist, and the latter should alert to the importance of considering individual differences in pursuing questions about childhood emotion reactivity and regulation.

Clustering patients by symptom-based diagnoses could lead to mixtures of individuals using different psychological and behavioural strategies. Treating all the members of mixed-strategy groups with the same technique(s) would be helpful to some and harmful to others. According to DMM based analyses, symptoms are seen as having evolved as part of the individual's adaptation to her circumstances, effective in eliciting a helpful response and so conceptualized as serving a function and maintained through the response of the system within which they are presented – including the health service. Underlying the symptoms are dispositional representations. Determining which DR drives the behavioural strategy, is crucial to identifying where to direct treatment.

The current findings also underscore the need for further clinical research into the biobehavioural nature of psychological interventions (Cicchetti & Blender, 2006). There are now many efforts underway to test the hypothesis that psychological interventions may alter both behavioural and neuroendocrine processes. That is a significant line of investigation because it seeks to translate some of the more impressive animal findings on the role of early caregiving, and because it would help to provide a broader public health context to psychosocial interventions. Multiple candidate mechanisms may be regulated by stress exposure such as the HPA or HPT axes (Gunnar & Quevedo, 2007; Schore, 1994); these are natural targets for further developmental studies of intervention effects.

The findings in this thesis highlight the importance of taking into account family functioning, parental psychopathology and gender, when investigating neuroendocrine biomarkers associated with attachment strategies and symptoms of anxiety and depression in obese children. We present empirical evidence that the harnessing of such a complex, mutually reinforcing dynamic across generations may open the way to the identification of relevant neuroendocrine biomarkers. How this may contribute to developing differentiated treatment approaches remains to be explored.

CHAPTER IX

PAPERS

Paper I

- **Pinto I.,** Wilkinson S., Virella D., Alves M., Calhau C., Coelho R. Anxiety, family functioning and neuroendocrine biomarkers in obese children. Acta Med Port. 2017; 30(4): 273-280.

Paper II

- **Pinto I.,** Wilkinson S., Virella D., Alves M., Calhau C., Coelho R. Attachment Strategies and Neuroendocrine Biomarkers in Obese Children. Acta Med Port. 2016; 29(5):332-339.

Paper I

- **Pinto I.,** Wilkinson S., Virella D., Alves M., Calhau C., Coelho R. Anxiety, family functioning and neuroendocrine biomarkers in obese children. Acta Med Port. 2017; 30(4): 273-280.

Anxiety, Family Functioning and Neuroendocrine Biomarkers in Obese Children

Ansiedade, Funcionamento Familiar e Biomarcadores Neuroendócrinos em Crianças Obesas

ARTIGO ORIGINAL

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ABSTRACT

Introduction: This observational study explores potential links between obese children's cortisol, and parental mental state, family functioning, and the children's symptoms of anxiety and depression.

Material and Methods: A non-random sample of 104 obese children (55 boys), mean age 10.9 years (standard deviation 1.76), was recruited from a childhood obesity clinic. Obesity was defined as body mass index above the 95th age- and gender-specific percentiles. Neuroendocrine biomarkers were measured. Symptoms of anxiety and depression were assessed with self and parent-reported questionnaires (Anxiety, Depression and Stress Scales; Child Behaviour Checklist). Family functioning was assessed with parent-reported questionnaires (Family Adaptation and Cohesion Scales-III).

Results: A significant, negative correlation (r_s = -0.779; p = 0.003) between girls' cortisol and their parents' anxiety symptoms was found, limited to high functioning families. Boys scored significantly higher than girls on parent-reported internalizing symptoms but not on self-report. No association was found between cortisol in children and parental depressive symptoms.

Discussion: Whether the association between cortisol levels in obese children and parental mental health is effectively restricted to girls from high functioning families or is due to study limitations, requires further research. The lack of associations between cortisol in children and parental depressive symptoms, suggests a specific association between cortisol and parental anxiety symptoms.

Conclusion: These results highlight the importance of taking into account family functioning, parental mental state and gender, when investigating neuroendocrine biomarkers in obese children associated with symptoms of anxiety and depression.

Keywords: Anxiety; Biomarkers; Child; Hypothalamo-Hypophyseal System; Pediatric Obesity; Pituitary-Adrenal System; Stress Psychological

RESUMO

Introdução: Este estudo explora relações entre o cortisol em crianças obesas e o estado mental dos pais, o funcionamento familiar e os sintomas de ansiedade e depressão das crianças.

Material e Métodos: Uma amostra de conveniência de 104 crianças obesas (55 rapazes), com idade média de 10,9 anos (desvio padrão 1,76), foi recrutada numa consulta de obesidade infantil. A obesidade foi definida pelo índice de massa corporal acima do percentil 95 ajustado para idade e sexo. Foram medidos biomarcadores neuroendócrinos. Os sintomas de ansiedade e depressão foram avaliados com auto e hetero-questionários (*Anxiety, Depression and Stress Scales*; *Child Behaviour Checklist*). O funcionamento familiar foi avaliado através de questionários aos pais (*Family Adaptation and Cohesion Scales -III*).

Resultados: Observou-se uma correlação negativa significativa (r_s = -0,779; p = 0,003) entre o cortisol das raparigas de famílias funcionais e os sintomas parentais de ansiedade. Os sintomas internalizantes relatados pelos pais (mas não os auto-relatados) são mais intensos nos rapazes. Não foi encontrada associação entre o cortisol das crianças e os sintomas depressivos dos pais.

Discussão: Não é possível assegurar se a associação entre cortisol sérico nas crianças obesas e sintomas de ansiedade parental se restringe a raparigas de famílias funcionais ou se deve a limitações do estudo. Os achados sugerem uma associação específica entre o cortisol sérico nas crianças obesas e a ansiedade parental.

Conclusão: É importante considerar o género, o funcionamento familiar, o estado mental dos pais ao investigar as associações entre biomarcadores neuroendócrinos em crianças obesas e sintomas de ansiedade e depressão.

Palavras-chave: Ansiedade; Biomarcadores; Criança; Obesidade Pediátrica; Sistema Hipófise-Suprarrenal; Sistema Hipotálamo-Hipofisário; Stress Psicológico

INTRODUCTION

Symptoms of anxiety and depression are frequent in obese children,[1] especially in girls, who in addition have metabolic risk.[1-3] Anxiety, depression and obesity are recognized burdens in both human and economic terms, therefore, it is important to investigate the aetiological mechanisms involved.[4] Mental disorders, such as anxiety and depression, produce decrements in public health that are equivalent to those of other chronic physical diseases (e.g., angina, arthritis, asthma, and diabetes).[4] However, they are stigmatized and regarded as less important. In fact,

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it is not generally acknowledged that, when mental disorders coexist with these physical diseases, the decrease in public health scores is substantially greater than when they occur alone. Thus, a crucial implication follows, that primary health care providers should not ignore the presence of depression and/or anxiety disorders when treating patients with a chronic physical disease.[5]

Metabolic syndrome, of which obesity is a part, shares risk factors with disorders of anxiety and depression. On the other hand, anxiety and depression are also known to increase the risk of obesity. Such a mutually reinforcing relation can contribute to the association between anxiety and depression and coronary artery disease.[5]

Mothers with symptoms of anxiety and depression and their offspring tend to have similar neuroendocrine biomarkers, such as altered levels of cortisol, peripheral levels of dopamine and serotonin, right frontal electroencephalogram activation and vagal tone.[6] If a description of specific subgroups, such as patients that associate certain biomarkers with specific symptoms, based on such data was to emerge, then, both short and long term benefits of treatment could potentially be improved. Although new reports about treatment response in multisite studies have emerged in the past five years, treatment advances are lagging due to scarcity of adequate studies with the appropriate indicators of response.[5]

Neuroendocrine biomarkers, such as those currently associated with the future development of symptoms of anxiety and depression, seem more frequent in individuals coming from families characterized by intense anxiety and depression.[2] Moreover, boys and girls living in such families may show differences in their vulnerability to develop symptoms of anxiety and depression themselves. Therefore it is important to take into account the mental state of family members, family functioning and the child's gender, when investigating potential neuroendocrine biomarkers for future anxiety and depression symptoms as found with obesity.

Most strategies for the prevention of childhood obesity are focused on energy balance.[7] They are directed at behaviours and environments that directly affect energy intake or expenditure, such as increasing physical activity, reducing sedentary behaviour, or limiting intake of high energy foods and beverages.[8] However, the limited success of these strategies[9-11] underscores the importance of developing new approaches through identifying other barriers to successful public health strategies.

Given the current state of knowledge, it is reasonable to assume that internalizing symptoms, such as those found with anxiety and depression, may be risk factors for obesity in children.[12-14] Moreover, it also seems sound and acceptable to consider that obesity be mediated through effects on appetite, sleep, and activity.[12-14] The (re)activity of the hypothalamic–pituitary–adrenal (HPA)-system plays a role in the course of disorders of anxiety and depression. In the few studies that have investigated the association between anxiety disorders and cortisol levels in children and adolescents, findings have been as inconclusive as in adults. Del Giudice and Shirtcliff[6] hypothesized that stressful influences in early life may be associated with higher basal levels of cortisol and hyper-responsiveness to subsequent minor stressors provoke frequent elevations in cortisol. The resulting periods of high levels of cortisol are further hypothesized to lead to down-regulation of components of the HPA-system. Another possibility is that under repeated stress, the initial cortisol response becomes modified over the years, so that initial high responses subsequently become lower.[15] Based on both these possibilities, and on the assumption that long periods of stress in early life would also predispose to future vulnerability for anxiety, we infer that anxiety disorders can be expected to appear in association with lower basal cortisol levels.

Given the current lack of information about causal processes leading to the major health risks associated with both anxiety and depression, and obesity, further research is needed to facilitate the development of both testable hypotheses and the future research agenda. This study aims to explore associations between cortisol levels and symptoms of anxiety and depression in obese children. We hypothesize that gender and family functioning, as characterized by different levels of cohesion and adaptability, play a role together with cortisol levels and symptoms of anxiety and depression in obesity. We also considered whether parental mental state could be associated with family functioning and cortisol levels in obese children.

MATERIAL AND METHODS

A sample of children attending the Childhood Obesity Paediatric Unit of a tertiary paediatric hospital in Greater Lisbon, was drawn from pre-pubertal obese children recruited for the project Roots of Early Obesity.[16] The effective sample was based on the children for whom we had detailed records of anthropometric information and behavioural measurements throughout childhood. Exclusion criteria were the use of medication, having already reached puberty during the period of assessment, undergoing mental health intervention or other medical treatment besides those for obesity.

Anxiety and depressive symptoms were assessed using Anxiety, Depression and Stress Scales (EADS) such as 'EADS-C – *Escala de Ansiedade, depressão e stresse para crianças*', child-version; and 'EADS - *Escala de Ansiedade, depressão e stresse*', adult-version. EADS-C and EADS are self-report questionnaires validated for Portugal.[17,18] The EADS-C and EADS are a set of three self-reported scales (21-items) designed to measure symptoms of depression (dysphoria, hopelessness, devaluation of life, self-depreciation, lack of interest/involvement, anhedonia, and inertia), anxiety (autonomic arousal, skeletal muscle effects, situational anxiety, and feeling anxious) and stress (difficulty relaxing, hypervigilant, and being easily upset/agitated, irritable/over-reactive and impatient). The responses are classified according to four levels: 0) never happens; 1) happens sometimes; 2) happens very often; and 3) happens almost all the time. EADS-C has a structure

similar to the adult version[18] although the load values of the items in each dimension are less discriminative than in adults.[17]

The Child Behaviour Checklist (CBCL 6 – 18) is a parent-reported questionnaire for assessing behavioural or emotional problems in children and adolescents (aged 6 to 18 year olds). It contains 120 items on behavioural or emotional problems experienced in the six months prior to the test. These items are scored on a three point scale: 0 = not true; 1 = somewhat or sometimes true; 2 = very or often true.

The CBCL version used is approved by Achenbach (http://www.aseba.org/ordering/translations.html).[19] This instrument is used to explore internalizing and externalizing symptoms. As conceived by Achenbach (1991), internalizing symptoms refer to three categories: withdrawal, somatic complaints, and anxiety/depression. Externalizing symptoms refer to the categories of delinquent and aggressive behaviour.

Family functioning was assessed using Family Adaptation and Cohesion Scales (FACES) III,[20] a parent-reported questionnaire, validated for Portugal.[20] Only one parent per child participated, most often the mother. This self-reported 20-item Lykert type questionnaire evaluates two major dimensions (Cohesion and Family Adaptation) on a circumplex model, which is integral to the conceptualisation of functioning employed in FACES. Reliability for Cohesion is 0.78 and for Family Adaptation is 0.70.[20]

Cohesion is conceptualised as the emotional closeness that family members have to one another. Adaptability is conceptualised as the potential for change in family leadership, role relationship, and relationship rules. FACES assesses the degree to which family members are adaptive and connected to each other. The dimension of family adaptability is categorised into four 'levels' of functioning: rigid, structured, flexible, and chaotic. The two middle levels (structured and flexible) are considered as balanced family adaptability and the two extreme levels (rigid and chaotic) are considered as unbalanced levels of family adaptability. The dimension of family cohesion is categorised into four 'levels' of functioning: disengaged, separated, connected, and enmeshed. Similar to that for family adaptability, the two middle levels (separated and connected) are considered to be balanced levels of family cohesion and the two extreme levels (disengaged and enmeshed) are considered to be unbalanced levels of family cohesion.[20] In other words, balanced levels of cohesion and adaptability are conceptualised as reflecting healthier family functioning, and they are classified as 'high functioning families'. Unbalanced levels of cohesion and adaptability (very low or very high levels) are seen as reflecting more problematic family functioning, and they are classified as 'low functioning families'.

Height, weight, waist circumference, blood pressure and heart rate were measured following standardized protocols. Obesity was defined as BMI ($kg.m^{-2}$) above the 95th age - and gender-specific percentile.[21] Overweight was defined as BMI ($kg.m^{-2}$) above the 85th age- and gender-specific percentile.[21] Blood pressure was measured three times and the third reading was recorded.[21] Age and gender-specific percentiles were used.[21]

As part of the Roots of Early Obesity project,[16] fasting blood samples were drawn at 8 a.m. through an indwelling catheter, after 45 minuntes of rest. Plasma cortisol, glucose, insulin, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, triglycerides and catecholamines were measured using the standard procedures of the Clinical Pathology Laboratory of the institution.

Parental written, informed consent was obtained. The project was approved by the Hospital Medical Ethics Committee.

To investigate the nature of the relation between cortisol levels, and symptoms of anxiety and depression (modelled as ordinal variables), Spearman correlation analyses and significance tests were performed, both with respect to the global sample and to each of its above mentioned subgroups.[21] Data analysis was performed using the software SPSS 22.0.[22]

RESULTS

A sample of 104 recruited pre-pubertal children (55 boys), with mean age of 10.88 years (standard deviation 1.76) was included in the analysis. BMI was calculated for 63 children, of whom 59 were obese (35 boys) and four were overweight (one boy). Waist circumference was measured in 46 children, with median (P_{25} - P_{75}) of 90.5 cm (76.8 – 97.1). Hypertension was diagnosed in 13 out of 59 assessed children.

The values found for the symptom scores (anxiety, depression and internalizing symptoms, which included anxiety and depression in Achenbach's CBCL system) and

Table 1 - Symptom scores (anxiety, depression and internalizing symptoms) as median (P_{25} - P_{75}) for the whole sample

Measures	Symptom scores
EADS Anxiety symptoms n = 71	10.0 (2.0 - 17.0)
EADS Depression symptoms n = 71	13.0 (5.0 - 24.0)
EADS-C Anxiety symptoms n = 76	17.0 (2.5 - 45.5)
EADS-C Depression symptoms n = 76	20.0 (6.0 - 58.0)
CBCL Internalizing symptoms n = 65	11.0 (7.0 - 15.0)
CBCL Externalizing symptoms n = 65	9.0 (3.5 - 16.0)

EADS-C: *Escala de ansiedade, depressão e stresse*, child version; EADS: *Escala de ansiedade, depressão e stresse*, adult version; CBCL: Child Behaviour Checklist

Table 2 - Descriptives of the neuroendocrine and metabolic biomarkers values

Parameter (n)	Mean (SD)	Median	Min. - Max.	P_{25} - P_{75}
Glucose (mg/dL) (57)	92.5 (15.4)	93.0	1 - 120	89.0 - 98.0
Insulin (U/mL) (43)	14.55 (9.60)	13.4	0.28 - 52.95	7.99 - 16.92
Triglycerides (mg/dL) (51)	97.21 (39.80)	89.0	33 - 187	68.0 - 129.0
Cholesterol (mg/dL) (53)	161.85 (31.00)	166.0	49 - 227	145.0 - 184.0
HDL (mg/dL) (50)	48.80 (13.87)	47.0	24 - 91	38.0 - 57.3
LDL (mg/dL) (48)	107.93 (23.51)	109.5	59 - 167	95.0 - 118.5
ALT (U/L) (50)	28.20 (23.42)	23.0	11 - 167	17.0 - 30.0
AST (U/L) (48)	24.10 (7.44)	24.5	12 - 56	19.0 - 26.8
TSH (µIU/mL) (56)	2.74 (1.17)	2.6	0.72 - 5.92	1.8 - 3.5
T4 (ng/dL) (53)	1.27 (2.11)	1.0	0.59 - 16.20	0.8 - 1.2
ACTH (pg/mL) (68)	28.89 (20.67)	20.7	5.35 - 118.0	16.3 - 35.2
Cortisol(µg/dL) (53)	10.8 (5.16)	9.95	3.3 - 29.0	7.2 - 13.6
Dopamine urine (µg/dL) (18)	236.8 (128.07)	236.8	45.0 - 513.8	145.6 - 348.4
Dopamine plasma (µg/dL) (12)	115.6 (150.40)	37.3	6.0 - 510.96	24.8 - 205.40
Epinephrine urine (µg/dL) (17)	6.7 (7.36)	5.99	0.5 - 32.5	3.5 - 7.65
Epinephrine plasma(µg/dL) (12)	5.4 (9.66)	0.7	0.09 - 32.5	0.4 - 6.8
Norepinephrine urine(µg/dL) (17)	34.2 (25.46)	30.7	5.9 - 105.8	17.5 - 47.7
Norepinephrine plasma(µg/dL)(12)	15.8 (23.47)	5.8	0.8 - 81.4	2.4 - 19.1

Glucose: Basal plasma glucose; Max.: Maximum; Min.: Minimum; n: Number of assessed children; P: Percentile; SD: Standard deviation.

neuroendocrine and metabolic biomarkers in the complete sample are presented as Tables 1 and 2.

The distribution of symptom scores and serum cortisol levels in the whole sample showed no significant differences between either high and low functioning families, or between boys and girls, but the score (median, P_{25} - P_{75}) for internalizing symptoms (CBCL) showed a tendency to be higher in boys (12.5, 9.0 - 15.0 *vs* 9.0, 6.5 - 13.5; Mann-Whitney, $p = 0.066$) (see also Table 3). An interesting observation, which nevertheless lead to no changes in cortisol, was that parents of obese boys scored (median, P_{25} - P_{75}) markedly higher, as close to significance as it is possible to get, for depression symptoms (EADS) compared to parents of obese girls (28, 10 - 66 *vs* 10, 0-38; Mann-Whitney, $p = 0.051$).

Serum cortisol levels in girls had a significant ($p = 0.026$) but low ($r_s = -0.464$) negative correlation with parent-reported anxiety score (EADS) (Table 4). Nearly significant negative low correlations were found between serum cortisol levels and parent-reported anxiety score (EADS) in children from low functioning families, child-reported anxiety score (EADS-C) in children from high functioning families and the score for externalizing symptoms (CBCL) in girls (Table 4).

The distribution of symptom scores and cortisol levels according to gender and family functioning is presented in Table 1. Girls from low functioning families scored significantly lower for internalizing problems (CBCL) than boys from both high (Kruskal-Wallis, $p = 0.039$) and low (Kruskal-Wallis, $p = 0.018$) functioning families but scored similarly to females from high functioning families (Table 3). No significant differences were found when considering BMI categories.

Table 5 shows the results for the correlation analyses and significance tests between serum cortisol levels and symptom scores (anxiety, depression, internalizing and externalizing symptoms), for each of the four subgroups (according to gender and family functioning). There was a significant, negative correlation between obese girls' cortisol and their parents' self-reported anxiety symptoms limited to high functioning families ($r_s = -0.779$; $p = 0.003$). No association was found between child serum cortisol and parental self-reported depressive symptoms.

DISCUSSION

This study explores associations between basal serum cortisol levels and symptoms of anxiety and depression in a sample of obese children. We found a significant negative association between serum cortisol and the parents' anxiety symptoms, limited to girls and particularly significant for girls from high functioning families. Boys scored significantly higher for parent-reported internalizing symptoms than girls. Parents of obese boys scored markedly higher for symptoms of depression than parents of obese girls. No associations were found between the children's serum cortisol and their parents' depressive symptoms, indicating that the association that was found between serum cortisol and parents' anxiety symptoms was specific for anxiety.

One possible explanation for this association between parents' anxiety symptoms and children's cortisol levels is that it is secondary to alterations in HPA-system activity present from birth. HPA-system functioning is at least partially determined by genetic[23,24] and prenatal factors.[25] For example, corticotrophic releasing hormone (CRH) gene regulation involves multiple activating and repressing

Table 3 - Symptom scores (anxiety, depression and internalizing symptoms) and cortisol levels as median (P_{25} - P_{75}), according to gender and family functioning

Measures	High functioning family		Low functioning family	
	Boys	Girls	Boys	Girls
EADS Anxiety symptoms	10.0 (2.0 - 21.8) n = 18	7.0 (2.0 - 17.5) n = 17	14.0 (10.0 - 19.0) n = 11	3.5 (1.5 - 26.8) n = 6
EADS Depression symptoms	14.0 (4.5 - 32.3) n = 18	17.0 (2.5 - 24.0) n = 17	14.0 (6.0 - 21.0) n = 11	19.0 (0 - 44.5) n = 6
CBCL Internalizing symptoms	12.0 (9.5 - 14.5) n = 17	11.0 (8.0 - 16.0) n = 15	14.0 (9.0 - 22.0) n = 10	5.0 (2.0 - 7.5) n = 5
Cortisol (ug/dL)	8.5 (6.7 - 11.7) n = 14	10.6 (6.8 - 13.0) n = 13	11.2 (8.8 - 16.8) n = 9	15.2 (5.8 - 17.5) n = 4

EADS: *Escala de ansiedade, depressão e stresse*, adult version; CBCL: Child Behaviour Checklist

Table 4 - Correlation of cortisol levels (ug/L) with anxiety, depression, internalizing and externalizing symptoms, for each subgroup of the sample

	Boys	Girls	High functioning family	Low functioning family	Obese children
EADS-C Anxiety symptoms	r_s = -0.043 p = 0.832 n = 27	r_s = -0.149 p = 0.519 n = 21	r_s = 0.479 p = 0.071 n = 15	r_s = -0.308 p =0.356 n = 11	r_s = -0.191 p = 0.321 n = 29
EADS-C Depression symptoms	r_s = -0.080 p = 0.692 n = 27	r_s = -0.095 p = 0.683 n = 21	r_s = 0.355 p = 0.194 n = 15	r_s = -0.185 p =0.587 n = 11	r_s = -0.284 p = 0.136 n = 29
EADS Anxiety symptoms	r_s = 0.149 p = 0.442 n = 29	r_s = -0.464 p = 0.026 n = 23	r_s = -0.328 p = 0.102 n = 26	r_s = -0.485 p = 0.093 n = 13	r_s = -0.096 p = 0.628 n = 28
EADS Depression symptoms	r_s = 0.064 p = 0.742 n = 29	r_s = -0.016 p = 0.943 n = 23	r_s = -0.081 p = 0.692 n = 26	r_s = 0.103 p = 0.738 n = 13	r_s = 0.113 p = 0.565 n = 28
CBCL Internalizing symptoms	r_s = 0.001 p = 0.995 n = 27	r_s = 0.121 p = 0.583 n = 23	r_s = 0.331 p = 0.114 n = 24	r_s = -0.019 p = 0.950 n = 13	r_s = -0.080 p = 0.684 n = 28
CBCL Externalizing symptoms	r_s = 0.174 p = 0.386 n = 27	r_s = -0.375 p = 0.078 n = 23	r_s = 0.021 p = 0.924 n = 24	r_s = -0.225 p = 0.460 n = 13	r_s = -0.166 p = 0.399 n = 28

EADS-C: *Escala de ansiedade, depressão e stresse*, child version; EADS: *Escala de ansiedade, depressão e stresse*, adult version; CBCL: Child Behaviour Checklist; r_s: Spearman correlation coefficient; p: Significance level, n: Number of children per correlation

transcription factors, specifically the glucocorticoid receptors and cyclic adenosine monophosphate (cAMP). Another important biological aspect is timing in the transcription of the signals, which is critical for effective glucocorticoid (GC) repression of the cAMP induced CRH gene.[24] Therefore, differences in timing of stimulatory and repression signals may be important for adaptation of the organism to stress, thereby providing a molecular explanation for the variability in adaptation to stress. These factors may influence CRH secretion patterns,[26] feedback effects of cortisol on central GC receptors, or both.[24]

On theoretical grounds, one can expect alterations in HPA-system activity to be more evident in individuals with a long history of anxiety symptoms. These changes may be reasonably interpreted as expressing a state of accrued vulnerability which is akin to some increment in maternal stress, in particular, during the latter half of pregnancy. Such undesirable conditions are known to favour maternal weight gain during pregnancy.[27] An overactive HPA-system leading to elevated cortisol levels is associated with an increased prevalence of abdominal obesity, although previous studies have suggested that there are obese individuals who may have desensitization to glucocorticoids, thereby not exhibiting increased blood cortisol.[24] A well accepted hypothesis is that once the HPA-system is over-activated during early development, it remains permanently unstable, vulnerable or dysfunctional, possibly due to transcriptional/epigenomic mechanisms.[28]

These elevations could influence down-regulation of components of the HPA-system, as suggested by the Adaptive Calibration Model,[6] an evolutionary-developmental theory of individual differences in the functioning of the stress response system. HPA activity is regulated by a hierarchy of feedback loops at different levels in the axis, and the sensitivity of these feedback loops is a major factor in determining HPA responsivity. The HPA-system responds to chronic stressors with sustained cortisol elevation, resulting in a flattened diurnal rhythm of secretion. Chronic elevation is often followed by a negative rebound of the system to below the previous baseline level after the stressor terminates. This hypocortisolism phase can last months; its function is probably to facilitate recovery and offset the physiological and immune costs of high circulating cortisol.[6] Hence, high and persistent levels of anxiety could

Table 5 - Rank correlation analyses between serum cortisol levels and symptom scores (anxiety, depression, internalizing and externalizing symptoms), for each subgroups (according to gender and family functioning) of the sample

	High functioning family		Low functioning family	
	Boys	**Girls**	**Boys**	**Girls**
Anxiety symptoms (**EADS-C**)	r_s =0.546 p = 0.205 n = 7	r_s = 0.374 p = 0.362 n = 8	r_s = -0.132 p = 0.778 n = 7	r_s = -0.800 p = 0.200 n = 4
Depression symptoms (**EADS-C**)	r_s = 0.577 p = 0.175 n = 7	r_s = 0.195 p = 0.643 n = 8	r_s = -0.019 p = 0.968 n = 7	r_s = -1.000 - n = 4
Anxiety symptoms (**EADS**)	r_s = 0.151 p = 0.607 n = 14	r_s = -0.779 p = 0.003 n = 12	r_s = -0.313 p = 0.412 n = 9	r_s = -0.316 p = 0.684 n = 4
Depression symptoms (**EADS**)	r_s = 0.144 p = 0.624 n = 14	r_s = -0.293 p = 0.355 n = 12	r_s = -0.076 p = 0.846 n = 9	r_s = 0.738 p = 0.262 n = 4
Internalizing symptoms (**CBCL**)	r_s = 0.346 p = 0.247 n = 13	r_s = 0.347 p = 0.296 n = 11	r_s = 0.139 p = 0.721 n = 9	r_s < 0.001 p = 1.000 n = 4
Externalizing symptoms (**CBCL**)	r_s = 0.483 p = 0.094 n = 13	r_s = -0.474 p = 0.141 n = 11	r_s = 0.030 p = 0.940 n = 9	r_s = -0.316 p = 0.684 n = 4

EADS-C: *Escala de ansiedade, depressão e stresse*, child version; EADS: *Escala de ansiedade, depressão e stresse*, adult version; CBCL: Child Behaviour Checklist; r_s: Spearman correlation coefficient; p: Significance level; n: Number of children per correlation

be associated with low cortisol concentrations, reflecting resilience rather than a risk for psychopathology.[29]

This is in accordance with the present study´s findings for girls. We found a significant negative association between serum cortisol and parents' anxiety symptoms, limited to girls, and particularly significant for girls from high functioning families.

Family functioning might operate through either a threshold effect, or be secondary to reduced buffering effects of protective relationships.[7] Possibly parents with high levels of anxiety symptoms influence their children's vulnerability to anxiety symptoms through an excess of control. In fact, some overprotective parenting behaviour has been associated with increased risk for anxiety symptoms in children.[30,31] In the group from low functioning families, the association between parents' anxiety symptoms and cortisol was not found to be statistically significant. However, this limitation may well be just a reflection of the small sample sizes of children for whom complete data-sets were obtained (Table 5). It is also proposed that affects are 'infectious' – and mirror neurone influences may be at play so that the affective state of the parent is contagious. We already know that successful treatment of a depressive disorder in adolescents depends on treating any concomitant parental depressive disorder at the same time. But we are unaware of studies having looked at the same for anxiety.

There is another possibility to explain the slightly paradoxical finding of a relationship between higher functioning, parental anxiety symptoms and girls' cortisol. The findings depend on self-reports from the parents, and it may be that parents from lower functioning families, particularly those high on rigidity and disengaged, are less clear about reporting on their subjective affective states. The relation between family functioning and quality of the self-reported information needs further investigation. Nevertheless there is a definite relation between parental mental state and biomarkers in their children. Future research needs to look at whether there is a connection between parental mental state, parental awareness of their children's diverse forms of distress, including those of anxiety and depression, and family functioning. The finding about lower reporting of internalizing symptoms in girls from low functioning families, may also be a result of lower parental awareness of their daughters' subjective discomforts, rather than an absolute finding that these girls had actually less internalizing distress. Parents of obese boys scored markedly higher for symptoms of depression than parents of obese girls.

On the other hand, investigating the role of genetic, epigenetic, attachment strategies and their interactions was beyond the scope of the present study. The role of attachment strategies is addressed elsewhere.[32]

The areas of the brain that govern energy balance are also involved in the response to stress and in emotion regulation. Extreme and/or sustained stress is associated with disregulation of energy balance.[13,28,33] Protection against obesity may arise by improving children's ability to modulate their metabolic and behavioural responses to stress. Thus educated, they would be able to read their body signals, both implicit and explicit, and use the signals more appropriately to achieve a greater state of well-being, harnessing their needs. Children whose stress response is well regulated may be less likely, for example, to overeat in response to emotional distress, and may have longer sleep duration, which could also reduce their risk for obesity.[29,34]

Strengths and limitations

Our goal was to contribute to understand the complexity

of a wide range of associations with cortisol levels in obese children. The study was not designed to test hypotheses, include all possible relevant parameters or be designed to generalize the findings to other samples. Through identifying discrete subsamples it may be possible to identify factors that only have effects under limited circumstances. But our study size means that such subsamples were of necessity very small.

In this study, HPA-system activity was not directly assessed in response to a stressor. The results do not reflect absolute changes in cortisol levels but shifts in the diurnal rhythm which itself might not be constant when there is anxiety or depression. Thus, future studies that assess both measures simultaneously may reveal more differentiated, results. The ethical dilemma arise with exposing children to prospective stress, and it may be that one would have to be limited to such situations as arise with starting at a new school or similar.

The findings depend on self-reports primarily from the mothers, rather than both parents, and it may be that parents from lower functioning families are less clear about reporting on their subjective affective states. The FACES dimensions suggest different forms of low functioning, ie low in quality, but including dimensionally opposite extremes. The study cannot elaborate on this. Within the attachment paradigm we would expect the outliers to be a mixture of those who overemphasise their discomforts (Type C), and those who have always displayed little of their affective states, and been poorly aware of what they might be (Type A). The relation between family functioning and quality of the self-reported information underlines the importance of using multiple sources providing both self-report and observations, also while investigating neuroendocrine biomarkers of anxiety and depression.[35]

If specific subgroups based on patients in whom there are different associations between biomarkers and specific symptoms emerges, then, both short and long term benefits of treatment could potentially be improved.

Due to the exploratory nature of this project, it was only possible to identify a few discrete subsamples from which it seems possible to gain further insight into the role of factors that under certain circumstances contribute to the symptom constellation.

CONCLUSION

These results highlight the importance of taking into account family functioning, parental mental state and gender, when investigating neuroendocrine biomarkers associated with symptoms of anxiety and depression in obese children. We present empirical evidence that the harnessing of such a complex, mutually reinforcing dynamic across generations may open the way to the identification of relevant neuroendocrine biomarkers and, thereby, open the horizon for the design of effective preventive strategies.

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PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patient's data publication.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Paper II

- **Pinto I.,** Wilkinson S., Virella D., Alves M., Calhau C., Coelho R. Attachment Strategies and Neuroendocrine Biomarkers in Obese Children. Acta Med Port. 2016; 29(5):332-339.

ARTIGO ORIGINAL

Attachment Strategies and Neuroendocrine Biomarkers in Obese Children

Estratégias de Vinculação e Biomarcadores Neuroendócrinos em Crianças Obesas

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ABSTRACT

Introduction: Quality of the parent-infant relationship influences the mechanisms of development of the child's physiological stress regulation. This study explored associations between attachment strategies and both cortisol and thyroid stimulating hormone, hypothesized to be respectively a potential mediator and a potential intervening variable of the mother-child relationship in obese children.

Material and Methods: A sample of 83 obese children (46 boys), aged 10.9 (1.8) years was recruited from a child obesity clinic. Obesity was defined by body mass index percentile adjusted for age and sex. Metabolic biomarkers were measured by routine methods. Attachment strategies were assessed with self and parent-report questionnaires. Family functioning was assessed with parent-reported questionnaires (FACES-III). Multivariate linear regression analyses were performed.

Results: Type A, avoidant attachment strategies, had significant positive association with thyroid stimulating hormone levels and negative association with cortisol levels (R^2 = 0.352). Type B, secure attachment strategies, had significant positive associations with both hypothyroidism and body mass index percentile (R^2 = 0.541). "Insecure attachment" (types A and C combined) strategies showed some evidence of positive association with thyroid stimulating hormone (R^2 = 0.250).

Discussion: These findings suggest that there may be commonalities in the regulation of hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes. Processes involved in development of the type A attachment strategy appear to be associated with effects on the regulatory mechanisms of the hypothalamic-pituitary-adrenal axis.

Conclusions: In obese children, different attachment strategies are associated with diverse metabolic profiles. How this may contribute to developing differentiated treatment approaches remains to be explored.

Keywords: Child; Hypothalamo-Hypophyseal System; Object Attachment; Pediatric Obesity; Pituitary-Adrenal System; Stress, Psychological; Thyrotropin.

RESUMO

Introdução: A qualidade da relação pais-filho influencia o desenvolvimento do sistema fisiológico de regulação do *stress* da criança. Exploraram-se eventuais associações entre estratégias de vinculação e o cortisol e a hormona estimulante da tiróide, respectivamente como possíveis mediador e variável interveniente na relação mãe-filho, na criança obesa.

Material e Métodos: Foi recrutada na Consulta de Obesidade uma amostra de conveniência de 83 crianças obesas com 10,9 (1,8) anos de idade (46 rapazes). A obesidade foi definida pelo percentil do índice de massa corporal para idade e género e os biomarcadores neuroendócrinos foram medidos pelos métodos de rotina. As estratégias de vinculação foram avaliadas através dos questionários (IACA) para pais e crianças. O funcionamento familiar foi classificado através do preenchimento pelos pais do FACES-III. Foram analisados modelos multivariáveis de regressão linear.

Resultados: As estratégias de vinculação insegura do tipo evitante (tipo A) apresentaram uma associação significativa positiva com os níveis de hormona estimulante da tiróide e negativa com os níveis de cortisol (R^2 = 0,352). As estratégias de vinculação segura (tipo B) associaram-se positivamente ao hipotiroidismo e ao percentil de índice de massa corporal, ambas com significado estatístico (R^2 = 0,541). As estratégias de vinculação insegura apresentaram alguma evidência de associação positiva com a hormona estimulante da tiróide (R2 = 0,250).

Discussão: Estes achados sugerem a existência de factores comuns na regulação dos eixos hipotálamo-hipófise-adrenal e hipotálamo-hipófise-tiróide. Os processos envolvidos no desenvolvimento das estratégias de vinculação do tipo A parecem associar-se aos mecanismos regulatórios do eixo HPA.

Conclusão: Diferentes estratégias de vinculação estão associadas a diferentes padrões metabólicos em crianças obesas. Desconhece-se qual a sua contribuição para o desenvolvimento e diferenciação da abordagem terapêutica.

Palavras-chave: Apego ao Objecto; Criança; Obesidade Pediátrica; Sistema Hipotálamo-Hipofisário; Sistema Hipófise-Suprarrenal; Stress Psicológico; Tireotropina.

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INTRODUCTION

The child-parent relationship plays a major role in the child's early life, influencing socio-emotional development, emotion regulation abilities and leading to development of particular attachment strategies.[1-4] The quality of the relationship influences both behavioral and metabolic regulation systems as they are activated in stressful contexts.[5] Obese children's attachment strategies have not received much attention. There is evidence that variance in hypothalamic-pituitary-adrenal (HPA) axis activity varies with weight.[6] In the present study, the quality of attachment was examined in relation to various neuroendocrine biomarkers, including cortisol and thyroid stimulating hormone (TSH).

Methods for evaluating pre-pubertal children's attachment strategies have not been well developed, with the result that there is little information about any relations between metabolic indicators and attachment strategies in this age group. Hypotheses have been developed based on research in younger children.[7]

Secure attachment (type B) is associated with the child's approach to its mother at times of need, and is interpreted as basic trust in the mother's availability and responsiveness. The mother has the child 'in mind' and she is available at times of child stress. Through contingent responsiveness, she enables her child to regulate his state, first via her direct handling of the child and subsequently through enabling her child's self-contained management of his affect – an external process facilitates internalizing of the affect regulation. In contrast, the child of a consistently insensitive and unresponsive mother does not learn to expect its mother to be available in stressful situations and develops an insecure-avoidant attachment (type A). On the other hand, the inconsistently available mother elicits escalating displays of affect in her child, who forces his mother to respond on the basis of an intermittent reinforcement schedule, but remains ambivalent about close contact when proffered as it was only elicited under duress (insecure-resistant attachment, type C). The type A child focuses on the environment at the moment of reunion, ignoring the mother or appearing to approach her but with objects between them, preventing the distress of potentially not being acknowledged. The reunion behavior of the type C child is characterized by anxious contact seeking and clinging, while, at the same time, ambivalently resisting contact with his mother.

It appears that only little or no excess adrenocortical activation is observed in a securely attached child when separated from his mother.[5, 8,9] The findings in the insecure-avoidant and insecure-resistant attachment groups are inconsistent. In some studies, both insecure groups were found to have elevated cortisol levels,[8] others found increased cortisol levels only for insecure-resistant children.[10] These may to some degree reflect disagreements about how to classify particular behavioural strategies. Nevertheless it is theoretically coherent to expect elevated cortisol levels with type C, as those children are exposed to chronic unpredictable stress, their parents responding to their distress only intermittently. Such findings point to cortisol levels being, at least partly, affected by the caregiving environment.[11,12]

Evidence of the contribution of caregiving processes to the development and maintenance of obesity has been mixed.[6,13,14] The environmental factors contributing to cortisol reactivity and associated neuroendocrine changes have yet to be documented in people with obesity.[13] Subtyping insecure attachment may help explain variability in cortisol reactivity, since inter-individual variability in HPA-axis activity appears to vary with type A and C strategies.[15,16]

Cortisol, a product of HPA-axis activation, inhibits its own release through negative feedback actions at the level of the pituitary and other brain areas. Previous data indicate that glucocorticoids can inhibit the hypothalamic-pituitary-thyroid (HPT) axis, at the level of the hypothalamus and pituitary.[17] Because both the HPA and HPT axes are stress responsive endocrine systems, it has been of interest to determine if there is any cross-regulation or communication between the HPA and HPT axes.

There are few Portuguese studies correlating cortisol and TSH levels with neuropsychiatric conditions for children of this age. Some independent studies of Portuguese cohorts have been published relating interrelations between the pituitary-thyroid axis and major neurosignaling systems involved in schizophrenia's pathophysiology. The available evidence supports that thyroid hormones deregulation is a common feature in schizophrenia and the fine-tuning of crucial brain networks warrants thyroid hormones homeostasis.[17]

Obesity has been associated with changes in HPA and HPT-axis activity, attributed to disturbance of the feedback system.[18] The feedback loop is crucial in recovery from stress, which in turn is essential for healthy physiological and behavioral regulation.

The current study is part of the project "Roots of Early Obesity",[19] aiming at a wide ranging assessment of pre-pubertal obese children. We analyzed the hypothesis of an association between the strategies of attachment of obese children and their neuroendocrine biomarkers.

MATERIAL AND METHODS

Sample and procedure

A convenience sample of children attending the Child Obesity Paediatric Unit of a Tertiary Paediatric Hospital in Greater Lisbon, was drawn from pre-pubertal obese children recruited for the project "Roots of Early Obesity". Participants were those for whom detailed anthropometric information and behavioral measurements of the child's development were feasible to collect. Parental written, informed consent was obtained. The project was approved by the Hospital Medical Ethics Committee.

Measures

Attachment strategies

The attachment strategies of children were assessed

using "IVIA - inventário sobre a vinculação na infância e adolescência" (IACA – Inventory of Attachment in Childhood and Adolescence), a set of parent-reported and self-reported questionnaires, originally developed and validated in Portugal.[20] IVIA was designed to classify child-caregiver attachment strategies in childhood and adolescence, from 7 to 17 years of age. It categorizes attachment strategies as secure (type B), insecure-avoidant (type A), or insecure-resistant (type C). IVIA does not consider type D, insecure-disorganized/disoriented strategy,[21] following instead the dynamic maturational model of attachment and adaptation (DMM) classificatory system.[22,23] Satisfactory psychometric properties were reported.[20]

Family functioning

Family functioning and typology were assessed using FACES III (family adaptation and cohesion scale),[24] a parent-reported questionnaire, validated for Portugal.[25] Only one parent per child was surveyed, most often the mother. This self-reported tool is a 20-item Lykert type questionnaire that evaluates 2 major dimensions on the circumplex model: cohesion and family adaptation. Reliability for the cohesion is 0.78 and for the adaptability is 0.70.[25]

This tool assesses the degree to which family members are adaptive and connected to each other. The circumplex model is a classification system with 16 family types, organized in three general types: balanced, mid-range, and extreme. The family adaptability is classified in four levels: rigid, structured, flexible, and chaotic. The two central levels (structured and flexible) are considered as balanced family adaptability and the two extreme levels (rigid and chaotic) are considered as unbalanced family adaptability. The family cohesion is classified in four levels: disengaged, separated, connected, and enmeshed. The two central levels (separated and connected) are considered as balanced family cohesion and the two extreme levels (disengaged and enmeshed) are considered as unbalanced family cohesion.[24] According to the curvilinear hypothesis, balanced levels of cohesion and adaptability (low to high levels) tend to reflect healthier family functioning, while unbalanced levels of cohesion and adaptability (very low or very high levels) tend to reflect more problematic family functioning.[24] The general family types, determined by adaptability and cohesion scores, are balanced, mid-range, and extreme, according to Olson.[24]

Metabolic assessment

Height, weight, waist circumference, blood pressure and heart rate were measured following standardized protocols. Body mass index (BMI) ($kg.m^{-2}$) was calculated using height and weight. Obesity was defined as BMI ≥ 95th age and gender-specific percentile.[26] Blood pressure was measured three times and the third reading was recorded.[27] Age and gender-specific percentiles were used.[27]

Table 1 - Descriptives of the neuroendocrine and metabolic indicators

Parameter (n)	Mean (SD)	Median	Min.- max.	25th P - 75th P
Glucose (mg/dL) (53)	93.96 (9.356)	93.0	71 - 120	89.0 - 98.0
Insulin (U/mL) (39)	14.45 (9.85)	13.3	0.28 - 52.95	7.99 - 16.50
Triglycerides (mg/dL) (48)	97.5 (39.85)	89.0	33 - 187	68.0 - 129.75
Cholesterol (mg/dL) (50)	162.7 (31.52)	166.0	49 – 227	145.0 - 184.5
HDL (mg/dL) (47)	48.6 (14.24)	45.0	24 - 91	38.0 - 57.0
LDL (mg/dL) (45)	108.7 (23.78)	110.0	59 - 167	96.5 - 120.0
ALT (U/L) (46)	27.35 (23.86)	22.5	11 - 167	16.75 - 30.0
AST (U/L) (44)	23.75 (7.59)	23.5	12 - 56	18.25 - 26.0
TSH (µIU/mL) (52)	2.8 (1.21)	2.68	0.72 - 5.92	1.7 - 3.5
T4 (ng/dl) (49)	1.3 (2.19)	1.0	0.59 - 16.20	0.8 - 1.2
ACTH (pg/mL) (49)	26.4 (16.94)	20.65	5.35 – 105.0	16.6 – 33.2
Cortisol (µg/dL) (53)	10.7 (5.58)	8.8	3.3 - 29.0	6.7 - 13.5
Dopamine urine (µg/dL) (15)	248.0 (136.60)	240.85	45.0 - 513.8	155.4 – 354.0
Dopamine plasma (µg/dL) (11)	79.65 (88.49)	36.7	6.0 - 240.85	23.6 – 198.25
Epinephrine urine (µg/dL) (15)	6.5 (7.70)	5.99	0.5 - 32.5	3.4 - 6.99
Epinephrine plasma (µg/dL) (11)	4.5 (9.61)	0.48	0.09 - 32.54	0.3 – 6.1
Norepinephrine urine (µg/dL) (15)	34.6 (27.19)	28.1	5.9 - 105.8	15.9 - 56.2
Norepinephrine plasma (µg/dL) (11)	9.8 (11.68)	4.05	0.8 - 39.0	2.4 - 19.11

Glucose: basal plasma glucose; Max.: maximum; Min.: minimum; n: number of assessed children; P: percentile; SD: standard deviation

As part of the "Roots of Early Obesity"[19] project, fasting blood samples were drawn at 8 a.m through an indwelling i.v. catheter, after 45 min of rest. Children were considered healthy and had not taken any medication. Plasma cortisol, glucose, insulin, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, triglycerides, catecholamines, TSH and T4 were measured using the standard procedures of the Clinical Pathology Laboratory of the institution. TSH ≥ 3.1 μIU/mL was considered as laboratorial evidence of hypothyroidism.

Statistical analyses

To explore the association between attachment strategies (modeled as nominal variables), age, gender, family functioning and neuroendocrine and metabolic assessment measures, univariable linear regression models were applied.

Strategies types A and C were combined to provide an "insecure attachment" group (a standard practice using the Berkeley system for attachment classification associated with Mary Main), for multivariable analysis. Variables that had a significant association with each attachment strategy

Table 2A - Associations between attachment strategies (secure and insecure) and anthropometric, familiar and neuroendocrine variables (univariable analysis)

	Secure attachment (type B)		"Insecure attachment"	
	β-estimate (95% CI)	*p*-value	β-estimate (95% CI)	*p*-value
Gender (boys)	-0.007 (-0.095; 0.082)	0.882	0.072 (-0.069; 0.213)	0.309
BMI percentile	0.035 (-0.168; 0.237)	0.731	0.119 (-0.200; 0.439)	0.457
Family functioning (high level)	0.027 (-0.078; 0.131)	0.610	-0.012 (-0.179; 0.154)	0.883
Hypothyroidism	0.063 (-0.053; 0.180)	0.277	-0.001 (-0.186; 0.184)	0.989
TSH (μIU/mL)	-0.001 (-0.051; 0.049)	0.962	0.069 (-0.006; 0.144)	0.072
ACTH (pg/mL)	-0.003 (-0.005; 0.000)	0.061	-0.005 (-0.010; -0.001)	0.030
Cortisol (μg/dL)	0.002 (-0.008; 0.012)	0.672	-0.016 (-0.030; -0.002)	0.026

ACTH: adrenocorticotropic hormone; BMI: body mass index; CI: confidence interval; TSH: thyroid-stimulating hormone

Table 2B - Associations between attachment strategies (avoidant and resistant) and anthropometric, familiar and neuroendocrine variables (univariable analysis)

	Avoidant attachment (type A)		Resistant attachment (type C)	
	β-estimate (95% CI)	*p*-value	β-estimate (95% CI)	*p*-value
Gender (boys)	0.056 (-0.028; 0.140)	0.187	0.016 (-0.075; 0.107)	0.724
BMI percentile	0.141 (-0.050; 0.332)	0.144	-0.021 (-0.226; 0.183)	0.834
Family functioning (high level)	-0.051 (-0.153; 0.051)	0.316	0.039 (-0.066; 0.143)	0.456
Hypothyroidism	-0.012 (-0.122; 0.097)	0.820	0.011 (-0.112; 0.134)	0.855
TSH (μIU/mL)	0.048 (0.004; 0.091)	0.034	0.021 (-0.030; 0.073)	0.405
ACTH (pg/mL)	-0.003 (-0.006; 0.000)	0.032	-0.021 (-0.005; 0.001)	0.142
Cortisol (μg/dL)	-0.008 (-0.016; 0.001)	0.075	-0.008 (-0.017; 0.001)	0.069

ACTH: adrenocorticotropic hormone; BMI: body mass index; CI: confidence interval; TSH: thyroid-stimulating hormone

were included in multivariable explanatory models tested by multiple linear regression, in which gender, BMI percentile and the classification of family functioning were always included. A p - value ≤ 0.05 was considered statistically significant. Statistical analysis was carried out using Statistical Package for the Social Science for Windows version 22.0 (SPSS Inc, Chicago IL).

RESULTS

From 83 recruited pre-pubertal children, 73 children (40 boys) with all the required data were included. The sample had a mean age 10.86 (SD = 1.8) years. The BMI was above the 97th percentile in 68 children (93%). The mean waist circumference, measured in 34 children, was 88.1 cm (SD = 13.99 cm). Systolic blood pressure was above the 95th percentile in 20 out of 59 children (33.9%).

The distribution of the measurements of the neuroendocrine values and metabolic indicators is presented in Table 1.

From the univariable analysis between attachment strategies and the neuroendocrine and metabolic indicators, ACTH, cortisol, TSH and hypothyroidism were identified as potentially associated with secure attachment (type B), "insecure attachment" (Table 2A) and avoidant attachment (type A) (Table 2B). No association was found for resistant attachment (type C) (Table 2B).

The main results of multivariable analysis of the explanatory models for the attachment strategies are shown in Table 3. Type A, avoidant attachment strategies, had significant positive association with TSH levels and negative association with cortisol levels (R^2 = 0.352). Type B, secure attachment strategies, had significant positive associations with both hypothyroidism and BMI percentile (R^2 = 0.541). The "insecure attachment" strategies group showed some evidence of positive association with TSH (R^2 = 0.250). ACTH was not found correlated with any type of attachment. No explanatory model could be derived for type C, resistant attachment strategies (Fig.1).

DISCUSSION

Associations between neuro-endocrine and metabolic indicators and attachment strategies were explored in a convenience sample of pre-pubertal obese children from a child obesity clinic. Detailed somatic and behavioral measurements of the child's development and family functioning were collected.

Using multivariable analysis, we found evidence of the association between avoidant attachment strategies (type A) with higher TSH levels and lower cortisol levels. On the other hand, secure attachment strategies (type B) was associated with both the presence of laboratorial evidence of hypothyroidism and higher BMI percentile.

When investigating associations between metabolic indicators and attachment strategies, the role of family functioning and gender should be taken into account. Therefore, we investigated the role of, and interactions between, several biological and environmental factors to see if any specific sub-groups could be identified which may benefit for further description and specific treatment strategies. Type A strategies were found to exhibit a negative, strong association with cortisol levels (β = -0.015; p = 0.036; R^2 = 0.352). This finding offers evidence of an increased risk for dysfunctional HPA-axis in children that score high in type A attachment strategies.

As previously reported,[10] insecure attached infants (Berkeley system) have been found to display high cortisol levels after a stressful stimulus. This possibility remains and it seems to be the result of different classification procedures, notwithstanding the fact that this finding was not independently validated by other researchers.[29,30] The high activation of the attachment system of insecure children may not terminate soon after its reunion with the primary caregiver, because the child is unable to use effectively the attachment figure for its regulation, making it difficult for a state of homeostasis to be reached.[15]

Other explanation for these findings is that elevation of cortisol is associated with the presence of a concurrent affective disorder.[9] On the other hand, cortical suppression could be due to a pattern of stress habituation over time, a pattern that increases the risk of difficulties in emotional and behavioral regulation; equally, reduced stress responsiveness may emerge as a result of genetic factors, or GxE interactions.[31] These differing patterns may in part reflect adaptations of the HPA axis to different periods of onset and chronicity, and differential genetic susceptibility. Confounders, such as depression, which is a frequently observed comorbidity, may account for some of the reported differences.[32] It is of mention the lack of depressive symptoms investigation was a handicap, but it was beyond the scope of the present study. "Roots of Early

Table 3 - Adjusted association between attachment strategies and anthropometric, familiar and neuroendocrine variables (multiple linear regression analyses). No associations with resistant attachment (type C) were found.

	Associated variables	β-estimate (95% CI)	p-value	R^2
Avoidant attachment (type A)	Cortisol	-0.015 (-0.028; -0.001)	0.036	0.352
	TSH	0.084 (0.015; 0.154)	0.021	
Secure attachment (type B)	Hypothyroidism	0.207 (0.092; 0.321)	0.002	0.541
	BMI percentile	0.291 (0.104; 0.477)	0.004	
"Insecure attachment"	TSH	0.128 (0.000; 0.256)	0.050	0.250
	ACTH	-0.011 (-0.025; 0.002)	0.087	

Variables in the models: gender, BMI percentile, family functioning classification, hypothyroidism, cortisol, ACTH, TSH.
ACTH: adrenocorticotropic hormone; BMI: body mass index; CI: confidence interval; TSH: thyroid-stimulating hormone.

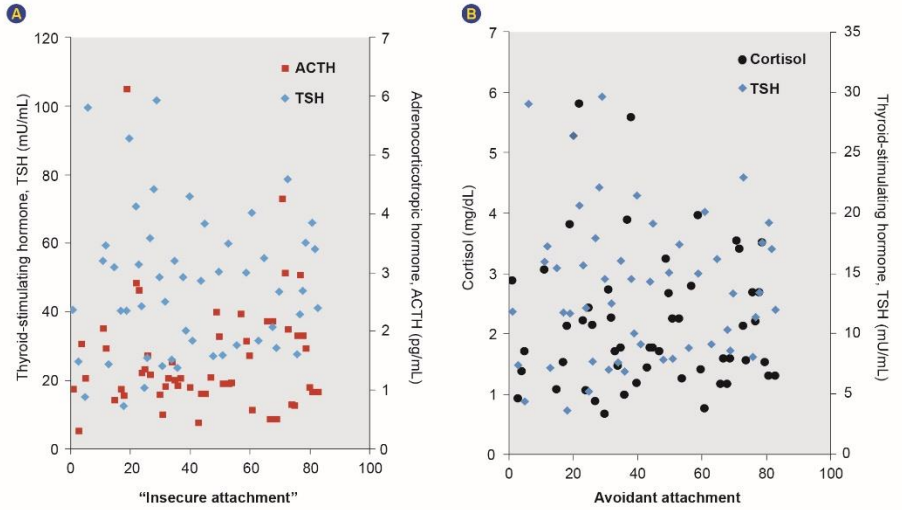

Figure 1 - Associations between insecure (A) and avoidant (B) attachment strategies and hormones of the hypothalamic-pituitary and the hypothalamic-pituitary-adrenal axis, relevant in the adjusted multivariate models

Obesity"[19] project explored depressive symptomatology and other comorbidities in a larger sample and this data will be submitted for publication subsequently.

It seems that childhood maltreatment may lead to atypical responsiveness of the HPA axis to stress, which in turn predisposes to psychiatric vulnerability in later life.[31] While there is general agreement around this broad principle, the putative mechanisms of how dysregulation of the HPA axis might mediate the link between stress and psychopathology and the precise nature of any interaction remains less clear.[32] It is possible that diminished cortisol responsiveness may emerge if early chronic stress leads to an initial hyper-activation of the HPA system which then progresses over time to a state of hyporeactivity, as a form of adaptation following sustained exposure to ACTH.[33]

In this study, HPA-axis activity was not assessed in response to a stressor given the ethical and practical implications of pharmacological challenge tests with children. As ACTH was not found altered in the explored models, cortisol changes may reflect differences in perceived acute stress which were not considered in the present study. Future studies that assess both measures simultaneously may reveal more differentiated results.

This study has the strengths of obtaining accurate biological and psychometric measurements, using validated instruments on a homogeneous sample of obese, pre-pubertal children for adjusted, multivariable analysis of associations. It has the limitation of using a rather small, convenience sample of children, based on a reference outpatient clinic for obesity, where most of the children have BMI above the 97th percentile. In spite of being a common distribution in non-clinical populations,[28] the small number of children affects mostly the power to identify significant associations with biological and psychometric variables alike. Therefore, the significant associations found in this sample should be considered as reliable. The small discriminant coefficients (R^2) for the models point out the multifactorial nature of the attachment strategies. Some of the etiological factors have not been collected and others were not found significant due to the sample size. It is impossible to determine the direction of the bias (if any) due to analyzing only those children for whom it was possible to collect all relevant information.

It is well known and accepted that the negative feedback system is essential in recovery from stressful situations and that a balanced stress recovery system that promotes homeostasis is of great importance. Presumably, an insecure attachment relationship does not facilitate adequate termination of the stress reaction. Now, this lack of homeostasis could put the child at metabolic and developmental risk, as long term negative outcomes have been shown to result from both insecure attachment[35-37] and childhood obesity. It should be noted, however, that the presently reported associations are merely correlational, and that the mechanisms underlying them need elaboration. In obese infants, these associations remain uncharted.[28]

CONCLUSIONS

These findings suggest that there may be commonalities in the regulation of HPA- and HPT-axes and/or communication between the axes. A common factor, such as developmental processes associated with

development of the type A attachment strategies, may be important in a linkage between the two axes. Furthermore, the correlation between the HPA and HPT axis may indicate that activity of one axis alters the activity of the other axis. As mentioned above, previous work has demonstrated that glucocorticoids, the end product of HPA axis activation, can inhibit the HPT axis.[38] This study supports this proposition, i.e., that lower cortisol may be associated with higher TSH levels.

The meaning of this for developing differentiated treatment approaches remains to be explored. Processes involved in development of the type A attachment strategy appear to be associated with effects on the regulatory mechanisms of the HPT axis. Indirectly this clear demarcation of the metabolic profile and attachment strategy is a validation of this Portuguese instrument for assessing attachment, but the study should be repeated using other measures of attachment strategy in this age group.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patient's data publication.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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CHAPTER X

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APPENDIXES

- Escala de Ansiedade, Depressão e Stresse para crianças (EADS-C)
- Escala de Ansiedade, Depressão e Stresse para adultos (EADS)
- Child Behaviour Checklist (CBCL 6-18)
- Inventário sobre a Vinculação na Infância e Adolescência (IVIA)
- Family Adaptation and Cohesion Scale (FACES III)

EADS-C – 21

Por favor lê cada uma das afirmações abaixo e assinala 0, 1, 2, ou 3 para indicar quanto cada afirmação se aplicou a ti ***durante a semana passada***. Não há respostas certas ou erradas. Não leves muito tempo a indicar a resposta em cada afirmação.

	Não se aplicou nada a mim	Aplicou-se a mim algumas vezes	Aplicou-se a mim muitas vezes	Aplicou-se a mim a maior parte das vezes
1. Tive dificuldades em acalmar-me.	0	1	2	3
2. Senti a boca seca	0	1	2	3
3. Não consegui sentir nenhum sentimento bom. Por ex. Não consegui parar de chorar.	0	1	2	3
4. Senti dificuldade em respirar	0	1	2	3
5. Tive dificuldade em tomar iniciativa para fazer coisas. Por ex. Não me apeteceu ver televisão, estudar e nem jogar computador.	0	1	2	3
6. Tive tendência a reagir em demasia em determinadas situações. Por ex. apeteceu-me bater num(a) colega que não se calava na aula	0	1	2	3
7. Senti tremores. Por ex., nas mãos, nas pernas	0	1	2	3
8. Senti que estava a utilizar muita energia nervosa.	0	1	2	3
9. Preocupei-me com situações em que podia entrar em pânico e fazer figura ridícula. Por ex. Ter muito medo, ficar muito assustado e, todos os meus amigos perceberem e gozarem comigo.	0	1	2	3
10. Senti que não tinha nada a esperar do futuro. Por ex. Que nada do que eu sonho, se podia tornar realidade.	0	1	2	3
11. Dei por mim a ficar agitado.	0	1	2	3
12. Senti dificuldade em relaxar. Por ex. Não conseguia estar sentado, parado e quieto.	0	1	2	3
13. Senti-me desanimado/cansado e melancólico/tristonho.	0	1	2	3

	Não se aplicou nada a mim	Aplicou-se a mim algumas vezes	Aplicou-se a mim muitas vezes	Aplicou-se a mim a maior parte das vezes
14. Estive intolerante em relação a qualquer coisa que me impedisse de terminar aquilo que estava a fazer. Como por ex. Faltar a luz, não conseguir terminar o jogo de computador e, ficar muito irritado e resmungão.	0	1	2	3
15. Senti-me quase a entrar em pânico ou seja, tive medo e fiquei muito assustado.	0	1	2	3
16. Não fui capaz de ter entusiasmo por nada. Por ex. nem jogar computador ou ver televisão eu tinha vontade	0	1	2	3
17. Senti que não tinha muito valor como pessoa ou seja, senti-me pouco importante.	0	1	2	3
18. Senti que, por vezes estava sensível. Por ex. Tive muita vontade de chorar de repente.	0	1	2	3
19. Senti alterações no meu coração sem fazer exercício físico. Por ex., o coração começou a bater muito depressa, de repente	0	1	2	3
20. Senti-me assustado sem ter tido uma boa razão para isso. Por ex. Fiquei cheio de medo sem ter acontecido nada.	0	1	2	3
21. Senti que a vida não tinha sentido. Por ex. Parece que de repente, as coisas deixaram de valer a pena.	0	1	2	3
10. Senti que não tinha nada a esperar do futuro. Por ex. Que nada do que eu sonho, se podia tornar realidade.	0	1	2	3

EADS-21

Nome _____ Data __/__/__

Por favor leia cada uma das afirmações abaixo e assinale 0, 1, 2 ou 3 para indicar quanto cada afirmação se aplicou a si ***durante a semana passada.*** Não há respostas certas ou erradas. Não leve muito tempo a indicar a sua resposta em cada afirmação.

A classificação é a seguinte:
0 – não se aplicou nada a mim
1 – aplicou-se a mim algumas vezes
2 – aplicou-se a mim de muitas vezes
3 – aplicou-se a mim a maior parte das vezes

1	Tive dificuldades em me acalmar	0	1	2	3
2	Senti a minha boca seca	0	1	2	3
3	Não consegui sentir nenhum sentimento positivo	0	1	2	3
4	Senti dificuldades em respirar	0	1	2	3
5	Tive dificuldade em tomar iniciativa para fazer coisas	0	1	2	3
6	Tive tendência a reagir em demasia em determinadas situações	0	1	2	3
7	Senti tremores (por ex., nas mãos)	0	1	2	3
8	Senti que estava a utilizar muita energia nervosa	0	1	2	3
9	Preocupei-me com situações em que podia entrar em pânico e fazer figura ridícula	0	1	2	3
10	Senti que não tinha nada a esperar do futuro	0	1	2	3
11	Dei por mim a ficar agitado	0	1	2	3
12	Senti dificuldade em me relaxar	0	1	2	3
13	Senti-me desanimado e melancólico	0	1	2	3
14	Estive intolerante em relação a qualquer coisa que me impedisse de terminar aquilo que estava a fazer	0	1	2	3
15	Senti-me quase a entrar em pânico	0	1	2	3
16	Não fui capaz de ter entusiasmo por nada	0	1	2	3
17	Senti que não tinha muito valor como pessoa	0	1	2	3
18	Senti que por vezes estava sensível	0	1	2	3
19	Senti alterações no meu coração sem fazer exercício físico	0	1	2	3
20	Senti-me assustado sem ter tido uma boa razão para isso	0	1	2	3
21	Senti que a vida não tinha sentido	0	1	2	3

OBRIGADO PELA SUA PARTICIPAÇÃO

Questionário de Comportamentos da Criança
CBCL 6-18 (© T. M. Achenbach, 2001)

Tradução autorizada:
Miguel Gonçalves (U. Minho)
Pedro Dias & Bárbara César Machado (U. Católica Portuguesa)

Nome da Criança: _____

Data de Nascimento: __/__/____ Idade: ____anos

Sexo: Masculino ☐ Feminino ☐ Ano de Escolaridade : _____

Escala Preenchida por: Mãe ☐ Pai ☐ Outro:_____ ☐

Profissão do Pai (mesmo que actualmente não trabalhe): _____

Profissão da Mãe (mesmo que actualmente não trabalhe): _____

Data de Avaliação: __/__/____

Segue-se uma lista de frases que descrevem características de crianças e jovens. Leia cada uma delas e indique até que ponto elas descrevem a maneira como o seu filho(a) é ou tem sido durante os últimos 6 meses:

-Marque uma cruz (X) no 2 se a afirmação é MUITO VERDADEIRA ou é MUITAS VEZES VERDADEIRA em relação ao seu filho;

-Marque uma cruz (X) no 1 se a afirmação é DE ALGUMA FORMA OU ALGUMAS VEZES VERDADEIRA;

-Se a descrição NÃO É VERDADEIRA, marque uma cruz (X) no 0.

Por favor, responda a todas as descrições o melhor que possa, mesmo que algumas pareçam não se aplicar ao seu filho(a).

0= Não verdadeira
1= De alguma forma ou algumas vezes verdadeira
2= Muito verdadeira ou muitas vezes verdadeira

0	1	2	1	Age de uma maneira demasiado infantil para a sua idade
0	1	2	2	Consome bebidas alcoólicas sem o consentimento dos pais (descreva)_____
0	1	2	3	Discute muito
0	1	2	4	Não consegue acabar as coisas que começa
0	1	2	5	Não há muitas coisas de que goste
0	1	2	6	Faz as suas necessidades fora da casa de banho
0	1	2	7	É fanfarrão ou gabarola
0	1	2	8	Não consegue concentrar-se, não consegue estar atento(a) durante muito tempo
0	1	2	9	Não consegue afastar certas ideias do pensamento; obsessões ou cismas (descreva):_____
0	1	2	10	Não é capaz de ficar sentado(a) sossegado(a), é muito activo(a) ou irrequieto(a)
0	1	2	11	Agarra-se aos adultos ou é muito dependente
0	1	2	12	Queixa-se de solidão
0	1	2	13	Fica confuso(a) ou desorientado(a) (parece não saber onde está)
0	1	2	14	Chora muito
0	1	2	15	É cruel com os animais
0	1	2	16	Manifesta crueldade, ameaça ou é mau para os outros
0	1	2	17	Sonha acordado(a) ou perde-se nos seus pensamentos
0	1	2	18	Magoa-se de propósito ou já fez tentativas de suicídio
0	1	2	19	Exige muita atenção
0	1	2	20	Destrói as suas próprias coisas
0	1	2	21	Destrói coisas da sua família ou de outras crianças
0	1	2	22	É desobediente em casa
0	1	2	23	É desobediente na escola
0	1	2	24	Não come bem
0	1	2	25	Não se dá bem com outras crianças
0	1	2	26	Não parece sentir-se culpado(a) depois de se ter comportado mal
0	1	2	27	Tem ciúmes com facilidade, é invejoso(a)
0	1	2	28	Quebra as regras em casa, na escola ou noutros locais
0	1	2	29	Tem medo de determinados animais, situações ou lugares, sem incluir a escola (descreva):_____
0	1	2	30	Tem medo de ir para a escola
0	1	2	31	Tem medo de pensar ou fazer qualquer coisa de mal
0	1	2	32	Sente que tem de ser perfeito(a)
0	1	2	33	Sente ou queixa-se de que ninguém gosta dele(a)
0	1	2	34	Sente que os outros andam atrás dele(a) para o apanhar; sente-se perseguido(a)
0	1	2	35	Sente-se sem valor ou inferior aos outros
0	1	2	36	Magoa-se muito, tem tendência para acidentes
0	1	2	37	Mete-se em muitas lutas/brigas
0	1	2	38	Fazem pouco dele(a) frequentemente
0	1	2	39	Anda com outras crianças/jovens que se metem em sarilhos
0	1	2	40	Ouve sons ou vozes que não existem (descreva):_____
0	1	2	41	É impulsivo(a) ou age sem pensar
0	1	2	42	Gosta mais de estar sozinho(a) do que acompanhado(a)
0	1	2	43	Mente ou faz batota
0	1	2	44	Rói as unhas
0	1	2	45	É nervoso(a), irritável ou tenso(a)
0	1	2	46	Tem movimentos nervosos ou tiques (descreva):_____
0	1	2	47	Tem pesadelos
0	1	2	48	As outras crianças/jovens não gostam dele(a)
0	1	2	49	Tem prisão de ventre, obstipação
0	1	2	50	É demasiado medroso(a) ou ansioso(a)
0	1	2	51	Sente tonturas
0	1	2	52	Sente-se demasiado culpado(a)
0	1	2	53	Come demais
0	1	2	54	Cansa-se demasiado
0	1	2	55	Tem peso a mais

2

0= Não verdadeira
1= De alguma forma ou algumas vezes verdadeira
2= Muito verdadeira ou muitas vezes verdadeira

			56	Apresenta problemas físicos **sem causa médica conhecida**:
0	1	2	a	Dores (sem ser dores de cabeça ou de barriga)
0	1	2	b	Dores de cabeça
0	1	2	c	Náuseas, sente enjoos
0	1	2	d	Problemas com a vista (não incluindo problemas corrigidos por óculos ou lentes de contacto) (descreva):_____
0	1	2	e	Irritações de pele/Borbulhas ou outros problemas de pele
0	1	2	f	Dores de estômago ou cólicas
0	1	2	g	Vómitos
0	1	2	h	Outros problemas (descreva):_____
0	1	2	57	Agride fisicamente outras pessoas
0	1	2	58	Tira coisas do nariz, arranca coisas da pele ou de outras partes do corpo (descreva):_____
0	1	2	59	Mexe ou brinca com os seus órgãos sexuais em público
0	1	2	60	Mexe ou brinca demasiado com os seus órgãos sexuais
0	1	2	61	O seu trabalho escolar é fraco
0	1	2	62	Tem fraca coordenação, é desajeitado(a) ou desastrado(a)
0	1	2	63	Prefere andar com crianças/jovens mais velhos
0	1	2	64	Prefere andar com crianças/jovens mais novos
0	1	2	65	Recusa-se a falar
0	1	2	66	Repete várias vezes e com insistência as mesmas acções ou gestos; tem compulsões (descreva):_____
0	1	2	67	Foge de casa
0	1	2	68	Grita muito
0	1	2	69	É reservado(a), guarda as coisas para si mesmo
0	1	2	70	Vê coisas que não existem, que não estão presentes (descreva):_____
0	1	2	71	Mostra-se embaraçado(a) ou pouco à-vontade
0	1	2	72	Provoca fogos
0	1	2	73	Tem problemas sexuais (descreva):_____
0	1	2	74	Gosta de se "exibir" ou de fazer palhaçadas
0	1	2	75	É envergonhado(a) ou tímido(a)
0	1	2	76	Dorme menos que a maior parte das crianças
0	1	2	77	Dorme mais do que a maior parte das crianças, durante o dia e/ou durante a noite (descreva):_____
0	1	2	78	É desatento(a), distrai-se facilmente
0	1	2	79	Tem problemas de linguagem ou dificuldades de articulação das palavras (descreva):_____
0	1	2	80	Fica de olhar fixo e vazio
0	1	2	81	Rouba coisas em casa
0	1	2	82	Rouba coisas fora de casa
0	1	2	83	Acumula coisas de que não necessita (descreva):_____
0	1	2	84	Tem comportamentos estranhos (descreva):_____
0	1	2	85	Tem ideias estranhas (descreva):_____
0	1	2	86	É teimoso(a), mal-humorado(a) ou irritável
0	1	2	87	Tem mudanças repentinas de disposição ou sentimentos
0	1	2	88	Ama muito
0	1	2	89	É desconfiado(a)
0	1	2	90	Diz palavrões ou usa linguagem obscena
0	1	2	91	Fala em matar-se
0	1	2	92	Fala ou anda durante o sono (descreva):_____
0	1	2	93	Fala demasiado
0	1	2	94	Arrelia muito os outros
0	1	2	95	Tem birras, temperamento exaltado
0	1	2	96	Pensa demasiado em sexo
0	1	2	97	Ameaça as pessoas

3

0= Não verdadeira
1= De alguma forma ou algumas vezes verdadeira
2= Muito verdadeira ou muitas vezes verdadeira

0	1	2	98	Chupa no dedo
0	1	2	99	Consome tabaco
0	1	2	100	Tem dificuldades em dormir (descreva):_____
0	1	2	101	Falta à escola sem razão (por "vadiagem")
0	1	2	102	É pouco activo(a), vagaroso(a), tem falta de energia
0	1	2	103	É infeliz, triste ou deprimido(a)
0	1	2	104	É invulgarmente barulhento(a)
0	1	2	105	Consome drogas sem razões médicas (descreva):_____
0	1	2	106	Comete actos de vandalismo
0	1	2	107	Urina-se durante o dia
0	1	2	108	Urina na cama
0	1	2	109	Choraminga
0	1	2	110	Gostaria de ser do sexo oposto
0	1	2	111	Isola-se, não se mistura nem estabelece relações com os outros
0	1	2	112	É preocupado(a)
			113	Por favor indique outros problemas do seu filho(a) que não tenham ainda sido referidos:
0	1	2		_____
0	1	2		_____
0	1	2		_____

VERIFIQUE, POR FAVOR, SE RESPONDEU A TODAS AS QUESTÕES.
SUBLINHE AS QUE O(A) PREOCUPAM DE UM MODO PARTICULAR.

I

Por favor enumere os desportos favoritos do seu filho(a). Por exemplo: natação, futebol, patinagem, skate, andar de bicicleta, pesca, etc.

Tempo - Em comparação com outras crianças/jovens da mesma idade, passa aproximadamente quanto tempo a praticar cada um? (1 - Menos que a média, 2 - Médio, 3 - Mais que a média)

Competência - Em comparação com outras crianças/jovens da mesma idade, em que grau consegue sair-se bem em cada um (1 - Pior que a média, 2 - Médio, 3 - Melhor que a média)?

Não pratica nenhum desporto ☐

	Tempo				Competência			
Desportos	Não sei	Menos	Médio	Mais	Não sei	Pior	Médio	Melhor
a.		1	2	3		1	2	3
b.		1	2	3		1	2	3
c.		1	2	3		1	2	3

II

Por favor enumere os passatempos, actividades e jogos favoritos do seu filho(a) que não sejam desporto. Por exemplo: selos, bonecas, livros, piano, trabalhos manuais, cantar, etc. (Não inclua ouvir rádio ou ver televisão).

Tempo - Em comparação com outras crianças/jovens da mesma idade, passa aproximadamente quanto tempo a praticar cada um? (1 - Menos que a média, 2 - Médio, 3 - Mais que a média).

Competência - Em comparação com outras crianças/jovens da mesma idade, em que grau consegue sair-se bem em cada um (1 - Pior que a média, 2 - Médio, 3 - Melhor que a média)?

Nenhum passatempo, actividade ou jogo ☐

	Tempo				Competência			
Passatempos, actividades ou jogos	**Não sei**	**Menos**	**Médio**	**Mais**	**Não sei**	**Pior**	**Médio**	**Melhor**
a.		1	2	3		1	2	3
b.		1	2	3		1	2	3
c.		1	2	3		1	2	3

III

Por favor enumere quaisquer organizações, clubes, equipas ou grupos a que o seu filho(a) pertença.

Grau de actividade - Em comparação com outras crianças/jovens da mesma idade, em que grau é activo em cada um (1 - Menos activo, 2 - Médio, 3 - Mais activo)?:

Não pertence a nenhuma organização, clube ou grupo ☐

	Actividade			
Organização, clube ou grupo	**Não sei**	**Menos**	**Médio**	**Mais**
a.		1	2	3
b.		1	2	3
c.		1	2	3

IV

Por favor enumere quaisquer empregos ou tarefas do seu filho(a). Por exemplo: dar explicações, tomar conta de crianças, fazer a cama, etc.

Grau de competência - Em comparação com outras crianças/jovens da mesma idade, em que grau consegue desempenhá-los bem (1 - Abaixo da média, 2 - Médio, 3 - Acima da média)?

Não desempenha nenhuma tarefa ☐

	Competência			
Tarefas	**Não sei**	**Abaixo**	**Médio**	**Acima**
a.		1	2	3
b.		1	2	3
c.		1	2	3

V

5

1. O seu filho(a) tem aproximadamente quantos(as) amigos(as) íntimos(as)? (Não inclua irmãos ou irmãs)

Nenhum amigo ☐ 1 amigo ☐ 2 ou 3 amigos ☐ 4 ou mais amigos ☐

2. O seu filho(a) tem actividades com os amigos(as) fora das horas de aula aproximadamente quantas vezes por semana? (Não inclua irmãos e irmãs)

Menos que 1 vez ☐ 1 ou 2 vezes ☐ 3 ou mais vezes ☐

VI.

Em comparação com outras crianças/jovens da mesma idade, até que ponto o seu filho(a) consegue relacionar-se com as seguintes pessoas? (Responda da seguinte forma: 1 - Pior, 2 - Próximo(a) da média, 3 - Melhor):

Não tem irmãos ☐

	Pior	Médio	Melhor
a. Consegue relacionar-se adequadamente com os seus irmãos e irmãs?	1	2	3
b. Consegue relacionar-se adequadamente com outras crianças/jovens?	1	2	3
c. Consegue comportar-se adequadamente em relação aos pais?	1	2	3
d. Consegue divertir-se e trabalhar por si próprio(a)?	1	2	3

VII

1. Para crianças com 6 ou mais anos de idade - Relativamente a cada uma das disciplinas escolares da tabela, indique como têm sido os resultados a cada uma delas (0 - Maus resultados, 1 - Abaixo da média, 2 - Médio, 3 - Acima da média)

Disciplinas	Maus resultados	Abaixo da Média	Médio	Acima da Média
a. Português	0	1	2	3
b. Francês e/ou Inglês	0	1	2	3
c. Matemática	0	1	2	3
d. História	0	1	2	3

Outras disciplinas escolares - por exemplo: Físico-química, Biologia, Geografia, Educação Visual.

Disciplinas	Maus resultados	Abaixo da Média	Médio	Acima da Média
e.	0	1	2	3
f.	0	1	2	3
g.	0	1	2	3

2. O seu filho(a) frequenta algum estabelecimento ou classe de ensino especial?

Não ☐ **Sim** ☐
(Que tipo de estabelecimento ou classe? _____)

3. O seu filho(a) repetiu algum ano?

Não ☐ **Sim** ☐
(Qual e porquê? _____)

4. O seu filho(a) teve algum problema na escola, de aprendizagem ou outro?

Não ☐ **Sim** ☐
Que tipo de problema? _____

Quando começaram esses problemas? _____

Os problemas mencionados já acabaram? _____

O seu filho(a) tem alguma doença, deficiência física ou mental?

Não ☐ **Sim** ☐ (Descreva-a, por favor _____)

O que o(a) preocupa mais no seu filho(a)? _____

_____

_____

Por favor, descreva o que o seu filho(a) tem de melhor:

_____

_____

_____

_____

_____

_____

_____

_____

_____

7

Inventário sobre a Vinculação para a Infância e Adolescência – Versão de Hetero-Avaliação

(Marina Carvalho, Isabel Soares, & Américo Baptista, 2006)

Seguidamente vai encontrar um conjunto de afirmações que descrevem características que as pessoas podem apresentar. Leia cada uma delas e assinale com uma cruz o número que melhor descreve o seu filho/a sua filha, utilizando a seguinte escala:

1	2	3	4	5
Nunca	Algumas Vezes	Muitas vezes	Quase Sempre	Sempre

1. Preocupa-se se tiver que depender de outras pessoas	1 2 3 4 5
2. É difícil confiar totalmente nas outras pessoas	1 2 3 4 5
3. Para ele/ela, é mais importante conseguir coisas que manter relações com outras pessoas	1 2 3 4 5
4. Preocupa-se com a possibilidade de ser abandonado	1 2 3 4 5
5. Acredita que as outras pessoas gostam dele/a e o/a respeitam	1 2 3 4 5
6. Para ele/a, é difícil depender de outras pessoas	1 2 3 4 5
7. Gostava de ser mais próximo dos amigos	1 2 3 4 5
8. Gosta de se sentir próximo das outras pessoas	1 2 3 4 5
9. Preocupa-se com a possibilidade de ficar sozinho/a	1 2 3 4 5
10. Para ele/a, é bom estar próximo de outras pessoas	1 2 3 4 5
11. Preocupa-se com a possibilidade de não ser aceite pelas outras pessoas	1 2 3 4 5
12. Fica ansioso/a quando alguém se aproxima de mais dele/a	1 2 3 4 5
13. Prefere não mostrar os seus sentimentos	1 2 3 4 5
14. Sente-se à vontade se tiver que pedir ajuda a outras pessoas	1 2 3 4 5
15. As outras pessoas podem contar com ele/a quando lhe pedem ajuda	1 2 3 4 5
16. Sabe que as outras pessoas estarão presentes quando necessitar delas	1 2 3 4 5
17. Sente que pode contar com as outras pessoas quando necessitar	1 2 3 4 5
18. Preocupa-se que os amigos não queiram estar com ele/a	1 2 3 4 5
19. Para ele/a, é muito importante sentir-se independente	1 2 3 4 5
20. As outras pessoas afastam-se dele/a porque tenta estar demasiado próximo delas	1 2 3 4 5
21. Prefere não depender das outras pessoas	1 2 3 4 5
22. Quando mostra os seus sentimentos pelos outros, tem medo que não sintam o mesmo por si	1 2 3 4 5
23. Sente que os pais o/a compreendem	1 2 3 4 5
24. Prefere que as outras pessoas não dependam dele/a	1 2 3 4 5
25. Não sabe se pode depender de outras pessoas para o/a ajudarem quando for necessário	1 2 3 4 5
26. Torna-se facilmente dependente das outras pessoas	1 2 3 4 5
27. Pede conselhos a outras pessoas quando está preocupado/a	1 2 3 4 5
28. Não gosta de contar às outras pessoas o que pensa e sente	1 2 3 4 5
29. Preocupa-se por poder não impressionar as outras pessoas	1 2 3 4 5
30. Acredita que as outras pessoas o/a rejeitam se se comportar mal	1 2 3 4 5
31. Respeita os sentimentos dos outros	1 2 3 4 5
32. Pode contar com os amigos quando é necessário	1 2 3 4 5
33. As outras pessoas aceitam-no/a tal como é	1 2 3 4 5
34. Não vale a pena expressar os seus sentimentos	1 2 3 4 5
35. Confia nas suas capacidades	1 2 3 4 5
36. Expressa claramente o que pretende	1 2 3 4 5
37. Pergunta-se se os amigos gostam realmente dele/a	1 2 3 4 5

FACES – III (D.H. Olson, J. Portner, Y. Lavee)

Versão Portuguesa de A. L. Roma Torres, R. Curral e F. Dourado

1	2	3	4	5
Quase nunca	Uma vez por outra	Algumas vezes	Frequentemente	Quase sempre

AGORA DESCREVA A SUA FAMILIA:

1.1 Os membros da família pedem ajuda uns aos outros.
1.2 Para resolver os problemas são seguidas as sugestões dos filhos
1.3 Aprovamos os amigos de cada um dos membros da família
1.4 Os filhos têm um apalavra a dizer no que diz respeito à sua educação
1.5 Gostamos de fazer coisas com a nossa família mais chegada
1.6 Na nossa família pessoas diferentes agem como lideres
1.7 Os membros da nossa família sentem-se mais próximos de outros membros da família do que de pessoas de fora
1.8 A nossa família pode mudar a maneira de executar as tarefas
1.9 Os membros da família gostam de ocupar o tempo livre uns com os outros
1.10 Os pais e os filhos discutem castigos conjuntamente
1.11 Os membros da nossa família sentem-se muito próximos uns dos outros
1.12 Na nossa família são os filhos que tomam decisões
1.13 Quando a nossa família se junta para alguma actividade toda a gente está presente
1.14 As regras podem mudar na nossa família
1.15 Podemos facilmente pensar sobre coisas que a família possa fazer em conjunto
1.16 Podemos trocar a responsabilidade das tarefas domesticas de uma pessoa para outra
1.17 Os membros da família consultam outros membros da família sobre as suas decisões
1.18 é dificil identificar quem manda na nossa família
1.19 A união familiar é muito importante
1.20 É dificil dizer quem faz cada uma das tarefas domésticas

FACES – III (D.H. Olson, J. Portner, Y. Lavee)

Versão Portuguesa de A. L. Roma Torres, R. Curral e F. Dourado

1	2	3	4	5
Quase nunca	Uma vez por outra	Algumas vezes	Frequentemente	Quase sempre

IDEALMENTE, como gostaria que a sua família fosse:

1.21 Os membros da família deveriam pedir ajuda uns aos outros
1.22 Para resolver os problemas as sugestões dos filhos deveriam ser seguida
23.Deveríamos aprovar os amigos de cada um dos membros da família
24. Os filhos deveriam ter uma palavra a dizer no que respeita à sua educação
25. Devíamos gostar de fazer as coisas com a nossa família mais chegada
26. Na nossa família pessoas diferentes agem como lideres
27. Os membros da nossa família deveriam sentir-se mais próximos de cada um dos membros da família do que de pessoas de fora
28. A nossa família deveria mudar a forma de executar as tarefas
29. Os membros da família deveriam gostar de ocupar o tempo livre uns com os outros
30. Pais e filhos deveriam discutir castigos conjuntamente
31. Os membros da família deveriam sentir-se mais próximos uns dos outros
32. Os filhos deveriam tomar decisões na nossa família
33. Quando a nossa família se junta todos deveriam estar presentes
34. As regras deveriam poder mudar na nossa família
35.Deveríamos poder pensar facilmente em coisas para fazer em conjunto com a nossa família
36.Deveríamos poder trocar as tarefas de casa uns com os outros
37. Os membros da família deveriam consultar cada um os outros nas suas decisões
38.Deveríamos poder saber quem manda na nossa família
39.A união familiar deveria ser muito importante
40.Deveríamos poder dizer que tarefas domésticas cada um deve fazer

Inês Pinto • 2017

U.PORTO

FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO